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Effects of Monosodium Glutamate Wastewater on the Fish *Ctenopharyngodon idellus* and the Cabbage *Brassica capestris*

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Various treatment processes for the wastewater from the production of monosodium glutamate (MSG) have been researched in the last 15 yrs. These include continuous fermentation process for single cell protein (SCP) production the upflow anaerobic sludge blanket process (Cao et al. , 1992), an external air lift reactor process (Cheng et al., 1994), and the use of the hybrid cell from the protoplast fusion between yeasts (Cheng et al., 19921995). There were a total of 16 abstracts concerning MSG wastewater treatment published in the Chemical Abstracts in the last 15 years Fifteen of these papers were from China, and the other was by Isik (1984) from the UK. So many publications indicate that the problems of MSG wastewater has not yet been solved and still has a great attraction for researchers. To deal with the problems of the MSG wastewater treatment, the first step is to establish the water quality profile. Therefore, the levels of 21 metals present in the wastewater were measured and compared with those cited in 4 different water quality standards in this study. It was proven by this test that the untreated MSG wastewater had acute toxicity, which was demonstrated by the bioassay with the fish *Ctenopharyngodon idellus* (Sprague, 1970).

Photosynthetic bacteria (PSB) have various metabolic pathways for the degradation of organic wastes. They can grow in the circumstances either with light or without light and either in aerobic or anaerobic conditions. The biomass of PSB cells not only serves as a source of food, but also increases fish weight, increases egg production in hens and improves the quantity and quality of citrus fruit when applied as an organic fertilizer (Zhou, 1983). *R.. sphaeroides* is one of the PSB species often used in the treatment of organic wastewater (Cheng et al., 1992, 1994). In this research, when the untreated MSG wastewater was diluted ten-fold with tap water, it could be treated with the PSB *R. sphaeroides. The fish* meal made up of the wastewater treated with *R. sphaeroides* and soyabean milk promoted the growth of the fish *C. idellus.* The wastewater diluted with drinking water increased the germination rate of tetraploid vegetable *B. capestris* and the seedlings grew well. Clearly untreated MSG wastewater is toxic to the fish *C. idellus* , but when diluted 10-fold and treated with *R. sphaeroides*, it has a beneficial effect on the fry and vegetable.

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MATERIALS AND METHODS

The MSG wastewater samples were provided respectively by three mills located in Nanjing, Zhenjiang and Yizheng in Jiangsu Province, P.R. China. The strains of the photosynthetic bacteria *R. sphaeroides* was provided by the Shanghai Institute of Plant Physiology Academia Sinica. The fry of the fish *C.idellus* were taken from the Fishery Institute of Jiangsu Province, with an average length of 5.8 cm and average weight of 3.3 g. The seeds of the Chinese cabbage *R. capestris* were provided by the Vegetable Research Institute of Nanjing Agriculture University. The levels of 21 metals were analyzed with the Inductively Coupled Plasma Spectrometer (ICP), J-A1100, USA, its detection limit being µ g/mL. The analysis was carried out by the Center of Analysis and Measurement at Nanjing University. COD_{Cr} (chemical oxygen demand: tested by potassium dichromate), BOD_5 (5-day biochemical oxygen demand), DO (dissolved oxygen), LC_{50} (median lethal concentration), and TSS (total suspend solid) were measured according to APHA (1980). The reactor (Biostat B) for PSB to treat MSG wastewater diluted 10-fold with tap water was produced by B. Braun Company, Germany, 1 L volume. TN (total nitrogen) and TP (total phosphorus) were measured by an oxidation method ($K_2S_2O_8$). The culture medium prescription for *R. sphaeroides* was referred to by Ormerod (1961), 10 g dextrose and 5 g sodium acetate: were used as the carbon source. In the fish toxicity tests, the untreated MSG wastewater was diluted with tap water to five concentrations: 50%, 30%, 10%, 6% and 2%. The death rates of the fish *C. idellus* were recorded at 24. 48, 73 and 96 hrs respectively for each of the concentrations in the tank with 40 liters of diluted wastewater at 20 °C. . Ten fry were put in each tank and asymptotic method was used to calculate the concentrations of the MSG wastewater when 50% of *C. idellus* died (Sprague, 1970). The ten-fold diluted MSG wastewater was treated with *R. sphaeroides in* the B. Braun reactor with 12 hrs hydraulic retention time, 30 °C reaction temperature. 0.6 mg/L of DO, 7.0 pH value, and the concentrations of BOD_5 and COD_{Cr} of the intake being 10% of that of the original MSG wastewater.

RESULTS AND DISCUSSION

Both organic and inorganic polution parameters of the Nanjing (NJ), Zhenjiang, (ZJ) and Yizheng (YZ) MSG wastewater are shown in Table 1. The concentrations of the 21 metals given in Table 1 were determined through ICP analysis. They represent only some of the metals in the MSG wastewater. Of the three mills, the concentrations of all the organic pollutant parameters at ZJ mill were the highest. Probably this was due to the fact that the corn starch could not be digested thoroughly by the bacteria in the fermentation process.

The results in Table 2 show the values of the pollutants in the MSG wastewater is how many Times Higher than the Standard Values (THSV) cited in the 4 water quality standards. THSV was taken as a metal or other pollutant parameter, when the metal pollution situation was evaluated and the possibility of making use of

MSG wastewater was analyzed and discussed. THSV= the actual concentration of a pollutant in a sample of MSG wastewater / the concentration of the pollutant permitted in a standard.

Table 1. Wastewater quality parameters of the samples from the effluents

Category (mg/L)	Source of MSG wastewater NJ	ZJ	YZ	Category (mg/L)	Source of MSG wastewater NJ	ZJ	YZ
Al	1.38	0.90	0.87	Ni*	0.65	0.20	0.12
Zn*	5844	1.88	6.63	Sr	0.71	0.27	0.44
Ba	0.15	0.14	0.26	Ti*	0.02	N.D	0.02
Ca	134.80	57.2	95.50	V*	0.02	<0.002	0.002
Cd*	0.69	<0.002	<0.002	Mo*	0.34	<0.005	0.013
Co*	0.05	<0.003	0.013	Be	0.01	<0.001	<0.001
Cr*	0.14	0.20	0.08	Li	0.04	<0.006	0.006
Cu*	0.02	0.09	0.11	COD_{Cr}	38068	249000	88720
Fe*	199.90	21.00	109.80	BOD_5	11284	74000	57600
K	331.40	632.50	555.10	TN	3840	2202	2680
Pb*	0.30	0.02	0.03	TP	36.9	43	241
Mg	69.90	109.80	96.70	TSS	1567	12900	10973
Mn*	7.34	1.05	1.88	pH	2.4	3.0	3.3
Na	12600	2365	2300				

* heavy metal element. N.D: not detected. The detection limits for metal elements are μ g/ml. Each number is taken from 3 sample readings.

From Table 2 it can be seen that Fe in the NJ, ZJ and YZ samples had the highest THSV according to the Sanitary Standards for Drinking Water (GB5749-85). The Fe may originate from the construction material for fermentor and the vessel system. Fe can be dissolved into the reaction liquid from the surrounding containers when the pH < 4.0, and the pH values of the MSG; wastewater are usually below 4. The recommended adult daily dose of Fe is 10-18 mg. Excessive Fe intake may cause vomiting or acute general intoxication. The concentrations of 8 metals from the NJ, ZJ and YZ samples were compared with those described in the WHO Standards for Drinking Water Quality. These are shown in Table 2. All the total THSVs of the heavy metals from every sample were higher than those in the GB5749-X5 comparison. It is clear that the WHO Standards for Drinking Water Quality are more stringent for heavy metal pollution Zn concentration also appears in the WHO standard. It is reported that if the concentration of Zn in a beverage is over 99 mg/L, it may lead to toxic effects. Standards for Irrigation Water Quality (GB5084-92) state that salt concentration in irrigation water must be restricted to the range from 1000 to 2000 mg/L. But all the total concentrations of the 21 metals in the wastewater samples from the three mills were higher than the salt concentrations in GB5084-92 standard. It can be estimated that the metal salt concentrations must be much higher than 1000-2000 mg/L. The THVSs of the organic pollutants in the MSG wastewater of the three mills ail exceeded the limit appearing in the GB5084-92. The concentrations of heavy metals in the wastewater samples from all the three mills exceeded the limits set by the Standards for Fishery Water Quality (GB11607-89). The restrictive values for the control of water body eutraphication are 0.2-0.3 mg/L of total nitrogen (TN) and 0.01-0.02 mg/L of total phosphorus (TP) (Cao, 19831, but the concentrations of TN were 3840 for NJ, 2022 for ZJ and 2680 for YZ, and those of TP were 241, 43 and 36 mg/L respectively. Such high levels of TN and TP, from the MSG wastewater will inevitably lead to eutraphication.

Table 2. THSV for the pollutants of wastewater compared with the 4 standards

GB5749-85 — THSV** (time)

Item	NJ,	ZJ,	YZ
Al	6	4	3
Cd*	13	-	-
Cr*	2	3	1
Fe*	999	104	544
K	27	52	45
Mg	-	1	1
Mn*	146	20	37
Na	83	15	14
Ni*	2	3	1
Pb*	7	-	-

WHO (1971, Geneva) — THSV (time)

Item	NJ,	ZJ,	YZ
Ba	-	-	-
Ca	-	-	-
Cd*	68	-	-
Fe*	1998	209	1089
Mg	-	-	-
Mn*	146	20	37
Zn*	1168	-	-

GB5084-92 — THSV (time)

Item	NJ,	ZJ,	YZ
Cd*	37	-	-
Cr*	-	1	-
Pb*	7	-	-
Zn*	2921	-	-
Cu*	-	-	-
TP	3	3	23
Total salts1)	19192	3190	3161
CODCr	126 -253	830 -1660	295 -590
BOD5	74 -140	492 -925	383 -720
TSS	7 -15	64 -128	53 -106

GB11607-89 — THSV (time)

Item	NJ	ZJ	YZ
Cd*	137	-	-
Cr*	-	1	-
Pb*	5	-	-
Zn*	58493	18	5
Cu*	1	8	10
Ni*	12	3	1
TN2)	3840	2202	2680
BOD5	2256	14799	11519
TSS	156	1289	10792

*heavy metal element. **THVS: times higher than the standard value. 1) the total concentration of 21 metal elements (mg/L). 2) TN (total nitrogen) concentration (mg/L) instead of that of Kjeldahl-N

The experimental animal was the fish *Ctenopharyngodon idellus,* used for testing the Median Lethal Concentration (LC_{50}) for the untreated MSG wastewater discharged from the YZ mill. The values of LC_{50} of the MSG wastewater to the fish were 24-hr LC_{50} = 6.7%, 48-hr LC_{50} = 3.2%, 72-hr LC_{50} = 2.5% and 96-hr LC_{50} = 1.4%. These results show that only after being diluted about 71434-fold can the MSG wastewater discharged from the YZ mill be turned safe to the fish *C. idellus* (APHA 1980). It is impossible for a mill to use so much water for dilution and the cost is rather high.

The MSG wastewater diluted ten-fold with tap water was treated with *R. sphaeroides* in an automatic control reactor. The treated wastewater contained the cells of *R. sphaeroides* at the concentration of 10^8 cells/mL. The fry of the fish *C. idellus* were fed with a solution of 10% the *R. sphaeroides*-treated wastewater and 90% soyabean milk. Thirty days after hatching, the average body weight was 31.80

mg and the average length was 12.50 mm. 12.50mm. The fish were fed for 31 days at 25 °C: Ten fish were placed in every 500 ml beaker 0% the water was changed daily and 50 ml of fish food was given daily.

After 32 days, all the length growth rate, weight growth rate and survival rate of the fish fed with the mixtures of 90% soybean milk and 10% *R. sphaeroides*-treated wastewater were the highest among the groups. They were 3.15%, 11.35% and 93% respectively. On the other hand, all the fish groups fed on the mixtures showed the three parameters were not lower than or at least similar to those of the control group. This indicates that *R.sphaeroides* can promote the growth and survival of the fish *C. idellus.* This result agrees with that reported by Zhou (1993).

Table 3. Effects of mixtures of *R. sphaeroides*-treated wastewater and tap water on thegermination rates and growth *of B. capestris* L. ssp. Chinesis (L.) Makino

Component of the solution (%effluent + % tap water)	Germination rate (%)	Average height of seedling (cm)	Color of seedling	Non-wilting rate (%)
0% +100 %(as control)	90	5.8	light green	75
2.5% + 97.5%	90	5.4	dark green	100
5.0% + 95.0%	50	5.3	dark green	75
10.0% + 90.0%	25	5.0	gray green	50
100 % + 0%	0	--	--	--

The tetraploid seeds of the Chinese cabbage *B. capestris* L. ssp. Chinesis (L.) Makino were watered with a solution of 10% MSG wastewate treated with *R. sphaeroides* mixed with tap water. The germination rates and growth rates are shown in Table 3. This test was carried out at 25 °C , illumination 10000 lx. For each group, 100 seeds were sowed in a plastic mug, 10 cm in diameter and 8 cm high, with pores in the bottom. From Table 3 we can see that, of the four groups watered with the mixtures, the germination rate of the group watered with the mixture of 2.5% treated wastewater and 97.5% tap water was 90% in 5 days, the same as that of the plain tap water group. Seven days after the seedlings sprouted, the average height of the plants watered with 2.5% treated wastewater and 97.5% tap water was 5.4 cm, shorter than those watered with plain tap water (5.8 cm). However, twenty days after sprouting, all the plants in the former group grew normally, while 25% of those in the latter group wilted. This shows that *R. sphaeroides* can foster the growth of *B. capestris* L. ssp Chinesis (L.) Makino. When 100% treated wastewater containing the cells of *R. sphaeroides* was used to water the seeds, the germination rate was 0, indicating that it stops the vegetable seed germination.

In conclusion, the concentrations of metals in untreated MSG wastewater exceed the levels cited in all the four standards examined in this research. The untreated wastewater is acutely toxic to the fish (*C. idellus* and also inhibits the growth of *R. sphaeroides*. However, if it is diluted 10-fold and treated with *R. sphaeroides* the wastewater becomes biologically benificial to the fish and the vegetable. Treated wastewater can increase the survival rate and promote the growth of the fish *C. idellus*. It does not inhibit the germination of the seeds of the Chinese cabbage *B. capestris* L. ssp. Chinesis (L.) Makino, but, on the contrary, promotes its growth at a proper concentration. The results of this study have shown the method to treat MSG wastewater and to transform it into something useful.

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