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Biotreatment of Distillery Effluent Using *Aspergillus niveus*

J. Angayarkanni,[1] M. Palaniswamy,[2] K. Swaminathan[1]

[1] Department of Biotechnology, Bharathiar University, Coimbatore 641 046, India
[2] Department of Biotechnology, Karpagam Arts and Science College, Coimbatore 641 021, India

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Presently, there are 285 distilleries in India (A.I.D.A., 1995). The proportion of wastewater generated in these industries is nearly 15 times of the total alcohol production, which amounts to 27 billion litres (Chhonkar et al. 2000). The effluent is characterized by high BOD, COD and dark brown colour. The high oxygen demand is due to the presence of large amounts of organic and inorganic matter. The presence of a brown pigment, melanoidins, impart dark colour to the effluent (Kort 1979). Reducing the colour of the effluent in addition to the COD and BOD reduction, is essential before discharging the effluent into the environment. In conventional methods, the colour degradation is very low and even is increased during treatment due to repolymerization of compounds (Wolfrom et al. 1953). Recently, microbial treatment using bacteria and fungi have received considerable attention due to simplicity in operationand effectiveness (Wiłkie et al. 2000). Moreover, the biologically treated effluent could be used safely and effectively to increase the soil productivity (Chhonkar et. al. 2000). In the present study, *Aspergillus niveus*, a litter degrading fungi, was used for the treatment of distillery effluent to reduce the colour, COD and BOD. The culture conditions for effective treatment were optimized. The efficiency of the treatment process was tested against *Zea mays* PMZ 103 seeds in terms of vigour index, phytotoxicity and effluent tolerant index.

MATERIALS AND METHODS

The fungus, *A. niveus* was isolated from Western Ghat ecosystem, Coimbatore, Tamil Nadu, India, by nylon net litter bag technique (Palaniswamy, 1997). The fungus was grown on Czapek (Dox) agar slants (Purvis et al, 1974) for 4 days at 27°±2°C. The spore suspension was prepared with sterile distilled water containing tween 80 (0.1% w/v) and 0.9% (w/v) sodium chloride. The spore concentration in the suspension was adjusted to 10^6 spores per mL. The spore concentration was determined by counting an aliquot (0.1mL) of the suspension using a haemocytometer under a microscope (Smith et al. 1991). Distillery effluent was collected from Sakthi sugars Ltd., Erode, Tamil Nadu, India. The effluent was amended with the following nutrients (g/L) (PenaMiranda et al. 1996) before treatment: sucrose – 10.0 g/L, KH_2PO_4 – 1.0 g/L, $MgSO_4$ – 0.5 g/L

Correspondence to: J. Angayarkanni

and NH_4NO_3 – 1.8 g/L. The amended effluent was autoclaved at 120°C for 15 min and inoculated with spore suspension (10% v/v). At regular intervals, samples were withdrawn and analyzed for colour (PenaMiranda et al. 1996) and chemical oxygen demand (COD) (APHA 1989).

To determine the optimum treatment period, the effluent was treated with fungus for a period of 10 days. Samples were withdrawn at regular time intervals of 24 h and analyzed for colour and COD. To minimize the cost of effluent treatment various carbon sources *viz.* paddy straw, sugarcane bagasse, molasses and sucrose were tried for effluent treatment and optimum concentrations were determined. After fixing the carbon source (sugarcane bagasse and sucrose), KH_2PO_4, $MgSO_4$ and NH_4NO_3 at different concentrations were tried as nutrient supplements.

For lab scale treatment, the effluent (5 L) amended with optimum concentration of nutrients was treated with the fungus for the optimum treatment period of 4 days. After the treatment period, the pollution parameters *viz.* pH, colour, COD, BOD (APHA 1989) and reducing sugar content (Somogyi 1952) of the treated effluent were determined. For pilot scale treatment 50 L of effluent amended with nutrients was taken in clean, surface sterilized plastic drum and treated with fungi under optimum conditions. After treatment for 4 days, the effluent was analyzed for pH, colour, COD, BOD and reducing sugar content.

To assay the phytotoxicity, *Zea mays* PMZ 103 seeds, obtained from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, was used as test system. The phytotoxicity of the treated effluents were determined in terms of vigour index, per cent phytotoxicity, effluent tolerant index and chlorophyll content of the seedlings. The pollution parameters were analyzed by Duncan's Multiple Range Test (DMRT) (Duncan 1955).

To determine vigour index *Zea mays* seeds were germinated in Petri dishes (25 seeds/replicate) lined with germination paper (45 x 28 cm), soaked and irrigated with different concentrations (25, 50, 75 and 100% v/v) of effluents at 25°C in a BOD incubator (ISTA 1966). On the fifth day, vigour index was calculated by multiplying per cent normal germination by embryonic axis length (cm) (Abdul-Baki and Anderson 1973). For determining per cent phytotoxicity and effuent tolerant index, *Zea mays* seeds were sown in aluminium trays containing coarse sand with 5 cm spacing and irrigated with various concentrations (25, 50, 75 and 100% v/v) of the effluents. After 10 days, the seedlings were plucked out carefully, without damaging the root system. Height of the shoot system and the length of the root system was measured. Seedlings irrigated with tap water served as control. Per cent phytotoxicity and effluent tolerant index were determined by the formula proposed by Turner and Marshal (1972).

To analyze the chlorophyll content, 500 mg of fresh leaves were homogenized with 10 mL of 80% acetone and centrifuged at 2100 g for 10 min. The residue was again extracted with 6 mL of 80% acetone. The homogenates were pooled

and made up to 20 mL with 80% acetone. The optical density of the extract was measured at 645 and 663 nm in a spectrophotometer. The chlorophyll content was calculated by the formula: Total chlorophyll (mg/mL) = (0.0202 x OD at 645) + (0.00802 x OD at 663). The results were expressed as mg chlorophyll per gram oven dry tissue.

RESULTS AND DISCUSSION

Paddy straw, sugarcane bagasse, molasses and sucrose were tried as carbon sources for the growth of fungus in the effluent. The substrates were amended in the effluent (10 g/L) along with the nutrients, KH_2PO_4, $MgSO_4$ and NH_4NO_3 (PenaMiranda et al. 1996) and inoculated with the fungal spore suspension (10^6 spores/mL of effluent). The flasks were incubated at 30°C for 10 days. At two days interval, samples were withdrawn and analyzed for colour intensity and COD content. The results (Fig. 1a) revealed that in all the substrates tried, incubation for a period of 4 days yielded maximum reduction in colour and COD. So, 4 days of treatment period was maintained for further studies. Among the four carbon sources, sugarcane bagasse and sucrose were observed to have profound effect on the treatment of distillery effluent by *A. niveus* (Fig. 1b). While comparing the two natural carbon sources, paddy straw and sugarcane bagasse, sugarcane bagasse at 1% (w/v) concentration resulted in maximum removal of colour (37%) and COD (91.68%). This observation might be due to the fact that sugarcane bagasse with higher glucan content could facilitate profuse growth of the fungus, thus resulting in effective treatment of distillery effluent (Wiselogel et al. 1996). When comparing the two synthetic carbon sources, molasses and sucrose, sucrose at 0.5% (w/v) concentration was found to be effective in removal of colour (26%) and COD (91.89%). This might be due to higher concentrations of non-biodegradable compounds present in molasses that could inhibit the fungal growth (PenaMiranda et al. 1996). Based on these observations, sugarcane bagasse at 10 g/L and sucrose at 5 g/L concentrations were selected for further studies.

Varying concentration of the nutrients amendment in the distillery effluent was observed to have a significant impact on colour and COD removal of *A. niveus*. Potassium dihydrogen phosphate at a concentration of 1 g/L resulted in maximum reduction of colour (29%) and COD (96.18%) with sucrose amendment. Among various concentrations of $MgSO_4$ tried, 0.25 g/L of $MgSO_4$ resulted in maximum colour (30%) and COD (97%) removal. Ammonium nitrate at 1.8 g/L concentration was found to be effective in reducing the colour intensity (29%) and COD (96%). PenaMiranda et al. (1996) have reported that the optimum concentration of nutrients, KH_2PO_4, $MgSO_4$ and NH_4NO_3, were 1.0 g/L, 0.5 g/L and 1.8 g/L respectively which effected 68% colour removal and 72% COD removal by *A. niger* from molasses wastewater. DMRT analysis of the data on colour and COD removal with varying concentrations of nutrients showed that the optimum concentrations were KH_2PO_4 – 1 g/L; $MgSO_4$ – 0.25 g/L; NH_4NO_3 – 1.8 g/L (Table 1 & 2). When the experiments were carried out with optimum culture

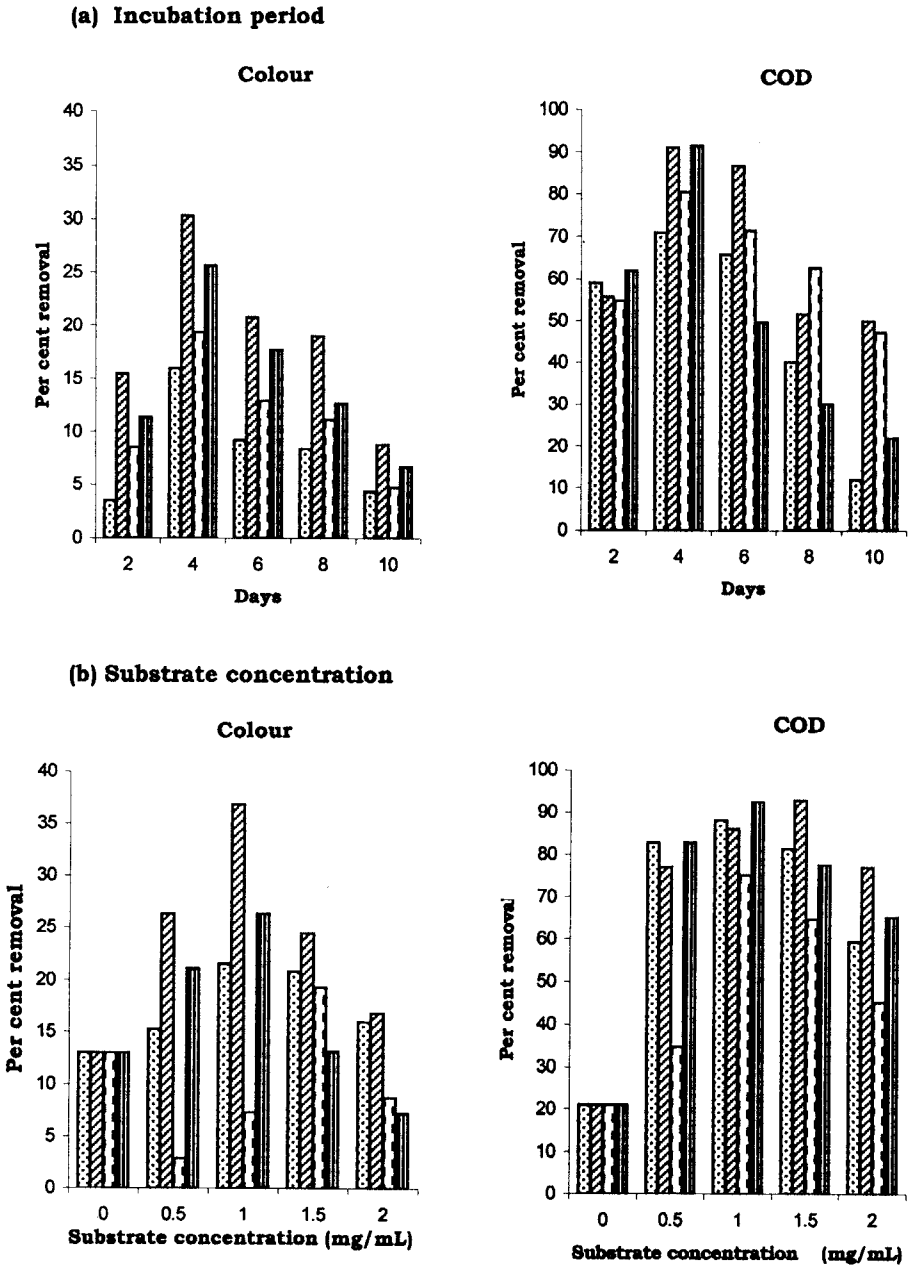

Figure 1. Optimization of incubation period and substrate concentration for the treatment of distillery effluent by *A. niveus*

Table 1. Effect of nutrients concentration on distillery effluent treatment using *A. niveus* (Incubation period : 4 days)

Concentration	Sucrose (1 % w/v)				Sugarcane bagasse (1 % w/v)			
(% w/v)	Colour	DMRT ranking	COD (mg/L)	DMRT ranking	Colour	DMRT ranking	COD (mg/L)	DMRT ranking
Potassium dihydrogen phosphate								
Untreated effluent	5.100	f,6	2,80,000	e,6	5.100	f,6	2,80,000	f,6
0.0	4.015	c,3	85,300	d,4	3.974	c,3	49,300	c,3
SD		0.002		7543		0.002		4989
0.5	3.724	b,2	53,300	b,2	3.794	b,2	34,700	b,2
SD		0.004		4989		0.002		4989
1.0	3.649	a,1	10,700	a,1	3.661	a,1	9,300	a,1
SD		0.001		1886		0.003		4989
1.5	4.107	d,4	69,300	c,3	4.128	d,4	74,700	d,4
SD		0.003		4989		0.004		4989
2.0	4.205	e,5	94,700	d,5	4.252	e,5	1,00,000	e,5
SD		0.002		4989		0.002		4989
CV:	1.0%		6.1%		1.0%		6.8%	
p:	0.01		0.01		0.01		0.01	
SED:	0.033		4.900		0.033		5.000	
LSD (1 %):	0.102		15.100		0.102		15.400	
Magnesium sulphate								
Untreated effluent	5.100	d,6	2,80,000	e,6	5.100	f,6	2,80,000	f,6
0.0	3.969	b,2	68,000	c,3	4.002	c,3	54,700	c,3
SD		0.001		6532		0.002		8219
0.5	3.608	a,1	5,300	a,1	3.532	a,1	8,000	a,1
SD		0.003		1886		0.002		5657
1.0	3.973	b,3	34,700	b,2	3.865	b,2	38,700	b,2
SD		0.002		4989		0.002		4989
1.5	4.098	c,4	73,300	c,4	4.177	d,4	85,300	d,4
SD		0.001		4989		0.001		6799
2.0	4.136	c,5	92,000	d,5	4.281	e,5	1,12,000	e,5
SD		0.001		3266		0.001		3296
CV:	1.0%		6.8%		1.0%		7.2%	
p:	0.01		0.01		0.01		0.01	
SED:	0.033		4.800		0.034		5.700	
LSD (1 %):	0.102		14.700		0.102		17.300	

SD – Standard deviation;The means followed by a common letter are not significantly different at 5% level

Table 2 . Effect of Ammonium nitrate concentration on distillery effluent treatment using *A. niveus* (Incubation period : 4 days)

Concentration	Sucrose (1 % w/v)				Sugarcane bagasse (1 % w/v)			
(% w/v)	Colour	DMRT ranking	COD (mg / L)	DMRT ranking	Colour	DMRT ranking	COD (mg / L)	DMRT ranking
Ammonium nitrate								
Untreated effluent	5.100	e,6	2,80,000	f,6	5.100	e,6	2,80,000	e,6
0.0	4.227	d,5	93,300	e,5	4.127	d,5	62,700	c,4
	SD	0.001		4989		0.001		8219
0.5	3.973	b,2	60,000	c,3	3.996	c,3	34,700	b,2
	SD	0.002		6532		0.003		4989
1.0	3.615	a,1	9,300	a,1	3.588	a,1	10,700	a,1
	SD	0.002		1886		0.003		4989
1.5	4.004	b,3	34,700	b,2	3.907	b,2	48,000	b,3
	SD	0.003		4989		0.002		6532
2.0	4.123	c,4	81,300	d,4	4.097	d,4	86,700	d,5
	SD	0.003		4989		0.001		8219
CV:	1.0%		6.2%		1.0%		8.9%	
p:	0.01		0.01		0.01		0.01	
SED:	0.033		4.700		0.033		6.300	
LSD (1 %):	0.102		14.300		0.102		19.200	

SD - Standard deviation; The values followed by a common letter are not significantly different at 5 % level by DMRT

conditions, sugarcane bagasse amendment yielded 35% reduction in colour and 95.25% reduction in COD; sucrose amendment yielded 43% and 96.68% respectively. After the short period of 4 days, the decolourization activity of the fungi got declined and colour intensity of the effluent was increased. This might be duc to the repolymerization of the coloured compounds present in the effluent (Sadahiro et al. 1988; Veronica at al. 1993). In lab scale experiment, 5 L of distillery effluent was treated with *A. niveus* under optimum conditions and after the treatment, the pollution parameters *viz.*, pH, colour, BOD, COD and reducing sugar content of the effluent were analyzed. Sugarcane bagasse was observed to be a better carbon source which reduced the pH to 8.1, colour by 60%, BOD by 96.39%, COD by 95.71% and reducing sugar content by 35.45%. Sucrose was found to be slightly less efficient than sugarcane bagasse and this treatment reduced the pH to 8.5, colour by 59%, BOD by92.34%, COD by 95.71% and reducing sugar content by 32.95%. Based on DMRT analysis, sugarcane bagasse

Table 3. Distillery effluent treatment using *A. niveus* (Incubation period : 4 days)

Fungus / Substrate	pH	Colour	DMRT ranking	BOD (mg/L)	DMRT ranking	COD (mg/L)	DMRT ranking	Reducing sugar	DMRT ranking
Labscale treatment									
Untreated									
effluent	9.0	5.100	b,3	10,000	c,3	2,80,000	b,2	0.0962	c,3
Sucrose									
(5 g / L)	8.5	2.094	b,2	766	b,2	12,000	a,1	0.0645	b,2
			0.03		1.00		1885		0.007
(10 g / L)	8.1	2.054	a,1	361	a,1	12,000	a,1	0.0621	a,1
			0.02		1.63		1885		0.029
CV:			1.9%		3.1%		3.9%		0.4%
p:			0.01		0.01		0.01		0.01
SED:			0.047		0.943		3.3		0.0002
LSD (1 %):			0.175		3.497		12.1		0.0008
Pilot scale treatment									
Untreated									
effluent	9.0	5.100		10,000		2,80,000		0.0962	
Sugarcane bagasse									
(10 g / L)	8.0	2.25		600		8,000		0.0624	
		(0.07)		(8.44)		(44.53)		(0.01)	
CV:			1.9%		3.1%		3.9%		0.4%
p:			0.01		0.01		0.01		0.01
SED:			0.041		1.356		3.93		0.0005

The means followed by a common letter are not significantly different at 5 % level by DMRT

was selected as carbon source for pilot scale studies. Pilot scale treatment was performed with 50 L of distillery effluent adapting the optimum conditions. The treatment resulted in the reduction of pH to 8.0, colour by 56%, BOD by 94%, COD by 97.14% and reducing sugar content by 35.14% (Table 3). Aoshima et al. (1985) reported a 75% reduction in colour of Baker's yeast factory effluent by *Coriolus versicolor* Ps4a after 4 days treatment. A two staged culture of sugarcane molasses stillage for SCP production of *Candida utilis* followed by *Paecilomyces variotii* resulted in a COD reduction of 92% (Lee and Baerwald 1991). *A. niger* was reported to reduce the colour by 68.5% and COD by 72.3% of molasses wastewater (PenaMiranda et al. 1996). A monoculture of *Hansenula* sp. on beet molasses stillage resulted in 35.7% COD reduction (Shojaosadati et al. 1998). White rot fungi, *Coriolus versicolor* was found to yield 71.5% colour removal along with a 90% COD reduction in anaerobically digested cane molasses stillage when the effluent was amended with glucose (Kumar et al.1998). When compared to these reports, the treatment processes tried in the

Table 4. Effect of treated distillery effluent on seed germination and seedling development of *Zea mays* test system

Treatment / Effluent concentration (%)	Per cent germination	Root length (cm)	Shoot length (cm)	Root biomass (mg)	Shoot biomass (mg)	Total biomass (mg)	Chlorophyll content (mg / g dry leaf)	Vigour index	Phyto toxicity	Effluent tolerant index
Control	100	21.33	33.67	0.0460	0.0667	0.1067	1.4243	868	0.000	1.000
Untreated effluent										
25	100	18.60	26.10	0.0380	0.0520	0.0900	1.4557	726	12.680	0.873
SD:		(1.100)	(1.012)			(0.002)	(0.049)	(12.02)		(1.012)
50	100	16.50	23.07	0.0247	0.0363	0.0610	1.2693	480	23.470	0.765
SD:		(1.050)	(1.131)			(0.003)	(0.073)	(6.01)		(1.132)
75	100	14.20	18.00	0.0187	0.0357	0.0544	0.7337	120	33.800	0.662
SD:		(1.072)	(0.899)			(0.004)	(0.019)	(3.12)		(1.268)
100	100	12.13	17.50	0.0177	0.0267	0.0444	0.3793	24	43.660	0.563
SD:		(1.050)	(1.723)			(0.003)	(0.063)	(0.600)		(1.334)
***A. niveus* treatment**										
25	100	21.57^{ns}	33.27^{ns}	0.0837	0.0757	0.1594	3.0513	882	0.000	1.000
SD:		(1.123)	(1.266)			(0.002)	(0.016)	(8.02)		0.000
50	100	21.70^{ns}	35.10	0.0760	0.0633^{ns}	0.1393	2.9257	580	0.000	1.000
SD:		(1.314)	(1.353)			(0.004)	(0.081)	(6.65)		0.000
75	100	18.50	26.10	0.0730	0.0517	0.1247	2.5860	380	13.150	0.869
SD:		(0.958)	(1.616)			(0.006)	(0.033)	(5.34)		(1.139)
100	100	14.60	15.27	0.0563	0.0413	6.0000	1.9663	140	31.460	0.685
SD:		(0.864)	(1.021)			(0.003)	(0.031)	(3.34)		(1.269)
CV:		1.9%	0.5%	2.4%	5.2%		0.1%	0.3%	0 .1 %	0.1%
p:		0.01	0.01	0.01	0.01		0.01	0.01	0.01	0.01
SED:		0.2896	0.1197	0.0013	0.0025		0.0012	1.1	0.0007	0.0009
LSD (1 %):		0.7903	0.3266	0.0036	0.0069		0.0032	2.9	0.0019	0.0024

ns: Not significant

SD : Standard deviation ; CV: Coefficient of variance; SED: Standard error deviation

present study were found to be very effective in COD (97.14%) and BOD (94%) reduction, but colour removal (56%) was lower. This decrease in colour removal might be due to the fact that the effluent taken for study was alkaline pH (pH 9.0) and the melanoidins responsible for the colour were more soluble in the alkaline pH. In the acidic pH, the melanoidins might be precipitated and removed easily (PenaMiranda et al. 1996). Though the optimum pH for the growth of *A. niveus* was 6.5 to 7.0 (Angayarkanni 2001), the fungus grew profusely at the effluent pH (9.0) also. Hence, the pH of the effluent was not reduced to minimize the cost of effluent treatment process. The treated effluent pH was within the limits (5.5 – 9.0) as specified by the Central Pollution Control Board of India (Chhonkar et al. 2000). Moreover, in this process, even the growth medium supplemented with nutrients is found to be effective. This will also reduce the treatment cost. Hence, this method could be considered as an economically cheaper method for treatment of distillery effluents.

Analysis of the toxicity of the effluents using *Z. mays* plant system revealed that both treated and untreated effluents were not toxic to seed germination, but seedling development was significantly inhibited by untreated effluent (Table 4). The diluted (25%) treated effluent stimulated seedling growth. The root and shoot length of the seedlings were slightly increased (1.7% and 4.25% respectively) by 50% (v/v) diluted treated effluent whereas, root biomass and shoot biomass were increased significantly 81.96% and 13.49% respectively by diluted (25% v/v) treated effluent. Even the undiluted treated effluent was found to increase the chlorophyll content of the seedlings. Though the untreated effluent was found to be non-toxic for seed germination, it had an adverse effect on vigour index, which was reduced by 97.23%. The treated effluent at 25% dilution did not affect the vigour index, but other concentrations were found to be inhibitory. Further, the data on phytotoxicity and effluent tolerant index showed that treated effluent up to 50% dilution was found to be non-toxic to seedling development of *Z. mays* seeds. The present study suggests that the litter degrading fungus, *A. niveus* could be used for treatment of distillery effluent by providing optimum nutrient conditions; the optimum conditions were 10 g/L of sugarcane bagasse as carbon source, 1 g/L of KH_2PO_4, 0.25 g/L of $MgSO_4$ and 1.8 g/L of NH_4NO_3 as additional nutrients with a treatment period of 4 days. The treated effluent after proper dilutions (up to 50%) could be used for irrigation of crop plants.

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