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Potentials of phototrophic bacteria in treating pharmaceutical wastewater

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ABSTRACT: A suspended growth photobioreactor was utilized to treat pharmaceutical wastewater by a wild strain purple non-sulfur photosynthetic bacterium isolated from the soil. The strain was named Z08 and identified as *Rhodobacter-sphaeroides* by 16SrDN. The photobioreactor was illuminated externally with two (40 W) fluorescent compact light sources on both sides. Its operation pH and temperature were between 6.8 – 7.0 and 20 – 30 ºC, respectively. Optimum growth of the isolate was obtained after enrichment of the pharmaceutical wastewater with 0.5 % ammonium sulfate and 0.1 % yeast extract under microaerobic optimum light (6000 lx) condition at 5d retention. Using these optimum conditions, the maximum dry cell weight and chemical oxygen demand percentage removal were 880 mg/L and 80 %. Chemical analysis of the culture after treatment of the enriched and non-enriched wastewater showed the crude protein content of the biomass to be 54.6 % and 38.0 %, respectively. This study proved that photosynthetic bacteria could transform complex wastewater that contains recalcitrant organic compounds with a resultant recovery of useful products.

Keywords: *Biotreatment; Microaerobic light; Photosynthetic bacteria; Purple non sulfur bacteria; Static light exposure reactor*

INTRODUCTION

Characteristics of the pharmaceutical industries are the diversity of their process operations, which gives rise to a wide variation in the liquid wastes. There is little similarity between effluents from different factories and individual effluents may differ continually as a result of process changes. In many cases, these effluents contain little or no biodegradable organic matters and the pollutant loads in terms of biological oxygen demand (BOD) may be negligible hence higher chemical oxygen demand (COD) than BOD (Bitton, 2005; Huseyin *et al.*, 2006). Most substances found in a pharmaceutical industrial wastewater are structurally complex organic chemicals that are resistant to biological degradation (Cokgor *et al.*, 2004; Ren *et al*, 2008), thus the need for segregation and collection of particularly toxic materials in a conventional biological treatment of pharmaceutical industrial wastewater (Gallely *et al.*, 1977).

Conventional biological treatment methods are usually inappropriate for the treatment of pharmaceutical wastewaters because of the negligible BOD content of the wastewater. For instance, the wastewater for this study has a BOD of 146mg/L and COD of about 8000 mg/L, resulting in the BOD/COD ratio of about 0.02. Hence, the need to try a novel biological treatment method which is the application of purple non-sulfur photosynthetic bacteria (PNSB) in degrading pollutants found in the pharmaceutical wastewater.

PNSB are widely distributed in the ocean, lakes, rivers, soil and activated sludge also in high temperature, low-temperature, low-salt, high-salt environment. They grow in both microaerobic and anaerobic light conditions while utilizing various substrates as sources of carbon and energy with ammonium and /or nitrate as a source of nitrogen and may also use sulphide or thiosulphate as an electron donor under photosynthetic conditions (Imhoff and Trüper, 1989; Kantachote *et al.*, 2005). They are metabolically the most versatile among all prokaryotes; anaerobically photoautotrophic and hotoheterotrophic in the light or microaerobic light condition (Holt *et al.*, 1994). Photosynthetic bacteria B) for wastewater

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treatment has generally proven to be a cost effective system for treating wastewater. This is because PSB does not only produce quality effluent, but produces substances of commercial interest such as single cell protein (SCP), biopolymers, antimicrobial agents and therapeutic compounds etc (Bertling *et al.*, 2006; Lorrungruang *et al.*, 2006).

Due to these properties, PNSB have been utilized by other researchers to treat different types of wastewaters such as concentrated latex wastewater (Choorit *et al.*, 2002), odorous swine wastewater (Myung *et al.*, 2004), tuna condensate (Prasertsan *et al.*, 1997), oil-containing sewage wastewater (Takeno *et al.*, 2005) and latex rubber sheet wastewater (Kantachote *et al.*, 2005). Kasomu and Obst (2009) studied the influence of photosynthesis on calcite precipitation.

The main aims of this study are to isolate, identify and test the ability of wild strain PNSB as well as to determine the optimum conditions for this isolate to be most effective in treating pharmaceutical wastewater. There could be another advantage in utilizing this PNSB to treat pharmaceutical wastewater because the biomass of PNSB has been reported to be a source of SCP production and could be used as an alternative to manure, fish feed, or agricultural supplement as it is rich in protein and vitamins (Getha *et al.*, 1998; Banerjee *et al.*, 2000; Kantachote *et al.*, 2005), hence the harvested biomass from the treatment will be analytically evaluated to ascertain it's suitability as a nutritional supplement.

This research was carried out between March 2008 and March 2009 at the School of Municipal and Environmental Engineering Department of Harbin Institute of Technology, China.

MATERIALS AND METHODS

Bacterium and culture

PNSB isolation

A purple non-sulfur bacterium named Z08 and isolated from soil was used to treat pharmaceutical wastewater. The bacterium was isolated with Sistrom minimal medium (RCVBN) (Eloi *et al.*, 1992) consisting of (in g/L) DL-malate: (4). $MgSO_4$: (0.12). $(NH_4)_2SO_4$: (1). $CaCl_2$: (0.075). KH_2PO_3: (0.3). Na_2 EDTA: (0.020). $VB_{1:}$ (0.001). Nicotinic acid: (0.001). Biotin: (0.015). Trace element solution, 1mL. The trace element solution contained (in mg/L) Zn $SO_4.7H_2O$: (20). $MnCl_2.4H_2O$: (6). H_3BO_3: (60). $CoCl_2.6H_2O$: (40). $CuCl_2.2H_2O$: (2). Na_2MoO_4: (6). 2g of soil was added to 95 mL sterile medium and incubated anaerobically at 28-35 ºC depending on the ambient temperature for about 96hrs under illumination with a 100W incandescent lamp. Anaerobic condition was created by addition of 5ml sterile liquid paraffin on top of the solution in the test tube. Purification of single colonies was achieved by successive re-streaking on the RCVBN medium containing 1.5 % agar and incubated at the same condition. The initial pH of the medium was adjusted to 6.8 by 5M NaOH.

Bacterial identification

The isolate was characterized using both morphological and physiological properties and according to Bergey's manual of systematic bacteriology (Imhoff and Trüper, 1989) and Bergey's manual of determinative bacteriology (Holt *et al.*, 1994).

Isolation and identification of bacteriochlorophylls

Bacteriochlorophylls were isolated, characterized and identified as described by Oelze (Okubo, *et al*, 2006). The isolated bacteria spectra were obtained with an Aminco DW-200 UV-Visible spectrometer in the split mode.

Cellular fatty acids analysis

Photosynthetic isolates were cultured on bactotryptic soy broth agar (Difco) and incubated at 28 ºC for 48h, the cellular fatty acids contents were determined using the MIDI procedure (MIDI, Inc, New York Del). Identification of the isolates were based on a comparison of fatty acid profiles using the fatty acid profiles in tryptic soy broth agar anaerobe database (Sasser, 1990).

Inoculums production

The inoculums were prepared by growing the cells in a modified Sistrom's minimal malate medium (pH 6.8) microaerobically in a reciprocating thermostatic shaker (unitron, Infors AG, Germany) at 200 rpm for 48 h with a temperature range of 28-30 ºC. Ammonium sulfate (0.99 g/L) and malate (4.02 g/L) were used as sources of nitrogen and carbon, respectively. Sterilization of the medium, photobioreactor and wastewater-test-media were accomplished by autoclaving at 121ºC for 15min, inoculation into the medium or bioreactor was as 10 % v/v inoculums from a culture growing in the modified RCVBN medium. For use as an inoculums, cell

suspension was adjusted to the desired optical density (0.500) at 660 nm using the sterile RCVBN medium as diluents. The sterile RCVBN medium was also used as the blank.

Wastewater

Wastewater from a pharmaceutical company located in Harbin, Heilongjiang province, China was collected at different times from the company's holding tank, each collection was analyzed for COD, BOD, suspended solids (SS), total dissolved solids (TDS) and pH. The properties of the wastewater varied at each collection time, hence representative wastewater sample was centrifuged at 4000 rpm for 10 min followed by autoclaving at 121ºC for 15 min to achieve sterile condition prior to use as the growth medium. It is imperative that autoclaving could cause volatilization of fatty acids and H_2S as the Teflon screw cap was at that stage loosely closed and this apparently would affect both the COD and BOD values. For this reason, the COD and BOD values reported in this study were determined after centrifugation and autoclaving of the raw wastewater. The original pharmaceutical wastewater without any pretreatment (centrifugation/ autoclaving) had COD and BOD values of 9450 and 197 mg/L, respectively.

Optimization of wastewater composition and treatment conditions

Effect of added nitrogen source, ammonium or nitrate were added to the sterilized wastewater (SW) medium at varied concentrations of 0, 0.1, 0.2, 0.3 and 0.4 %. The wastewater pH of 6.6 was not adjusted as the optimum pH for growing most bacteria is in the range of 6.5-7.5 (Bitton, 2005; Cheremisinoff, 1996). Ammonium being the best nitrogen source was tested again at higher concentrations of 0 0.5, 0.75, 1.0 and 1.5% respectively. In most cases SW medium was used under microaerobic light (3500 lx) for 72 h.

SW medium supplemented with optimal concentration (0.5 %) of ammonium was used as the base control to evaluate the effect of added yeast extract (YE). Yeast extract is a complex natural material with high vitamin B level; it was tested for its effect on growth at the following concentrations; 0, 0.01, 0.05, 0.1, 0.2, 0.3 %. All the cultivations were grown three consecutive times for 72 hrs under microaerobic light conditions.

The effect of light intensity variation was investigated by growing the isolate in sterilized optimized wastewater (SOW); i.e. the wastewater was supplemented with 0.5 % $(NH_4)_2SO_4$ and 0.1% yeast extract, grown under light intensities of 500, 1000, 2000, 4000, 6000 and 8000 lumens, respectively. These variations were obtained by adjusting the distances between light sources and the reactor tubes. In all these, the temperature variation was same (20-31ºC).

Photobioreactor

A test tube of 100 mL with cellophane tapered edge to prevent volatilization was used as a bioreactor. Three treatments were set as follows: (SOW) as a control without inoculums; SOW plus 10 % inoculums and SW plus 10 % inoculums. The photobioreactor temperature was between 20-31ºC depending on the ambient temperature. Illumination was with 40 W compact fluorescent lamps at the desired lumens on both sides of the bioreactor. Cell growth, pH and COD were monitored at the end of each retention time of 3, 5 and 7 days of cultivation, respectively. Microaerobic condition was achieved by creating a head space in the PBR, no flushing with either argon or nitrogen gases and the cultivation condition was static-light exposure. The dissolved oxygen (DO) levels of all treatment were between 0.7-1.63 mg/L.

Analytical methods

The incident light intensity was measured by a TES-1330A digital light meter, while the functional groups were determined with GC-FTIR. Organic compounds in the wastewater were identified qualitatively using gas chromatography coupled with mass spectrometry (GC-MS). Instrumentation was a Hewlett packard (HP) 5890 Series II gas chromatograph, interfaced with a HP Chem-Station data system and linked to a HP 5972 mass selective detector operated in scan mode. Results are reported as lists of those compounds reliably and tentatively identified. It meant that the organic compounds identification were carried out by computer matching against a HP Wiley 275 library of 275,000 mass spectra combined with expert interpretation and that the identified compounds were not confirmed against their standard compounds because of the inability to get the standard compounds during the time of the experiment.

For the determination of the parameters, the cell suspensions were centrifuged at 4000 rpm for 20 min and cell pellets were used for the determination of dry cell weight and crude protein. The supernatant was

used for COD, organic compounds, SS and pH determinations. The bacterial cell concentration was determined by optical density at 660 nm (i.e.OD_{660}) using a UV-VIS spectrophotometer (Shimadzu UV-120). The cell dry weights were determined by centrifuging 10 mL aliquots of culture at 4000 rpm for 20 min and the cell pellets were washed properly with distilled water then filtered using 0.45 µm pore size, 47mm in diameter membrane filter paper to remove salts and non-cellular materials. Each loaded filter was dried at 105 ºC until the weight was invariant (about 72 h). The dry weight of the blank filter was subtracted from that of the loaded filter to obtain the dry cell weight (DCW). The OD_{660} value was converted to DCW concentration via proper calibration, (where 1.0 OD_{660} approximately equals 0.6g dry cell/L.).

Crude protein was expressed as percentage total nitrogen (% N) multiplied by 6.25. TDS, SS, BOD, COD and pH were measured according to standard methods (APHA, 1992). It is worthy to mention that the COD values quoted in this study were measured after centrifugation and sterilization of the wastewater by autoclaving. Trace metals were measured using a Perkin Elmer Optima 5300DV ICP aided by a WTW microwave digester (WTW is a supplier name that deals on electrochemistry instruments just like Hach or YSI).
Organic acids (butyric, propionic and acetic acids) and alcohols were determined using gas chromatography (GC-14B, Shimadzu, Tokyo, Japan) equipped with a flame ionization detector (FID). Samples were injected into a 15 m long capillary column with an internal diameter of 0.53 mm. Nitrogen was used as the carrier gas with a flow rate of 20 mL/min. The temperature of injector and detector was set at 220 and 230 ºC, respectively. The oven temperature was initially set at 110 ºC, increased from 110 to 200 ºC at a rate of 8 ºC/min and held at 200 ºC for 5 min. Liquid samples were centrifuged (4000 rpm for 10 min) and filtered (0.45µm membrane) prior to being injected into GC for analysis. Biomass productivity was calculated from the total volume of wastewater used and the total weight of biomass produced in the process after each retention time. The pH was measured using a digital pH-meter (Metrohm 620). Centrifugation was carried out in a thermostatic Sigma centrifuge (B. Braun, Melsungen, Germany). Since the properties of the wastewater varied at each collection time, representative sample was used for the analyses of the organic compounds, functional group, total nitrogen (TN) and organic acids present in the wastewater. All experiments were conducted in triplicate and mean value reported.

RESULTS AND DISCUSSION

Identification of the bacterium

The isolate was a non-motile, gram negative, oval rod, 0.25 µm wide and 1.50 µm long and reproducing by binary fission, internal photosynthesis membrane appeared as lamella. After growth under anaerobic photoheterotrophic conditions, cell suspensions were red and the absorption spectra of living cells suspension showed maxima at 370, 570, 800, 827 and 852 as shown in Fig.1. The two main peaks at 800 and 850 nm, which are closely related to bacteriochlorophyll a and b (characteristics of photobacteria pigment) as well as carotenoid, are characteristics of PNSB (Madigan *et al.*, 2000). By GC-FAME (Gas chromatography of fatty acid methyl ester) analysis the PNSB isolate was identified as being closely related to *Rhodobacter sphaeroides* strains with similarity indices of 0.85 ± 0.05. This also identifies the isolate as Rhodobacter sphaeroides (Imhoff and Trüper, 1989; Yegani *et al.*, 2005) and it is named *Rhodobacter spheroides* z08. The isolate grew with organic compounds or thiosulphate in minimal/basal medium with either $(NH_4)_2SO_4$ or $NaNO_3$ as a nitrogen source anaerobically or microaerobically in the light.

Wastewater

The results of characterization of pharmaceutical wastewater are shown in Tables 1 and 2. According to Table1, the wastewater contains recalcitrant organic compounds such as octadecane, heptacosane and octacosane which are resistant to biodegradation, also compounds like benzothiazole and enzenepropanamine are all toxic and lethal in nature. These identifications were made by GC-MS spectrometry and it is supported by GC-FTIR where three peaks were evident at 1304 cm^{-1} and 1600-1300 cm^{-1} (Fig. 2), again suggesting the presence of aliphatic amino salts and aromatic compounds respectively. All these compounds are resistant to biodegradation and accumulate in the environment with a resultant negative effect on the food web. Table 2 also shows that the wastewater is high in COD than BOD, hence low value of BOD to COD ratio (0.02). This again depicts that the wastewater contains high level of non biodegradable organic substances (Bitton, 2005; Banu *et. al*, 2007) and difficult to treat with conventional biological

Table 1: List of organic compounds tentatively identified in the pharmaceutical wastewater

Peak No.	Name of compound	$t_{R\ (min)}$	Molecular ion peak (m/z)	Fragment ion (m/z)
1	Benzenamine	15.082	121	120
2	Benzothiazole	24.818	181	181
3	1,2-Benzenedicarboxylic acid 1-Butanamine Benzenepropanamine	31.315	278	149
4	Desmethyldoxepin	34.082	101	44
5	Methylpent-4-enylamine	36.262	149	44
6	Nitro-L-arginine	38.066	265	44
7	1-Octaecanamine	38.388	99	44
8	Octadecane	39.414	219	44
9	Heptacosane	40.517	283	44
10	Octacosane	41.760	254	57
11		43.144	380	57
12		44.702	394	57

Table 2: Characteristics of the untreated and treated pharmaceutical wastewater (all in mg/L except pH)

Physicochemical	Untreated	Treated
pH		7.6±0.3
CODcr	6.6±0.2	1,526.0±23
Total suspended solids	8,480.0±932	170.0±1.6
Total dissolved solids	425.0±2.3	360.0±1.2
Total nitrogen	1,600.0±1.1	21.5±0.6
BOD5	533.7±0.9	26.0±0.2
Zn	146.7±0.3	0.033.0
Cd	0.056.0	ND
Pb	ND	ND
Iron	ND	0.0223
Mn	2.100	0.575
Cu	0.605	ND
Acetic acid	0.022	N/A
Propionic acid	422.7	N/A
Butyric acid	201.3	N/A
	304.5	

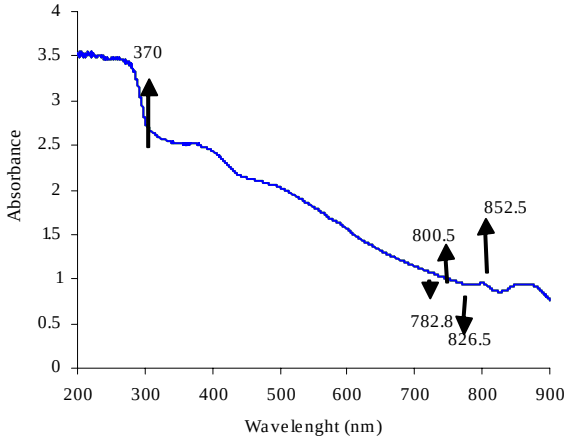

Fig. 1: In vivo spectrum of the isolate (pure culture)

treatment methods. These organic compounds found in the pharmaceutical wastewater could be vital part of everyday life, but most of them are of environmental concern because of their toxicity, persistence and tendency for bioaccumulation (Sapana *et al*., 2008). Some studies have demonstrated inhibition of enzyme activities with different pollutants, including hydrocarbons, heavy metals and surfactants (Martinez-Tabche *et al*., 1997; Rodriguez-Fuentes and Gold-Bouchot, 2000; Hosseini *et al*, 2007). All these contributed to the difficulty in conventional biological treatment of pharmaceutical wastewater, while being the major consideration for using PNSB to treat this wastewater. The GC-MS fragmentation analyses of the pharmaceutical wastewater after treatment of the optimized wastewater (Table 3) indicate that the PNSB was able to either ameliorate or transform those recalcitrant and xenobiotic materials found in the initial wastewater into some acceptable organic compounds. This finding is in support of Tchobanoglous *et al.*, (2003) which stated that complete biodegradation of recalcitrant organic compounds to harmless end products such as CO_2 and H_2O or methane may not always occur; instead biotransformation to different organic compound is possible. Those recalcitrant and xenobiotic compounds pose problems in conventional wastewater treatment, due to their resistance to biodegradation. This also shows that the PNSB used for this treatment is candidate for bioremediation since it could transform some toxic and recalcitrant organic compounds of natural origin found in the pharmaceutical wastewater. Takeno *et al*. (2005) treated oil containing sewage wastewater using photosynthetic bacteria; they recorded successful transformation of C 10- C 26 (hydrocarbon) by the photosynthetic bacteria.

Table 3: List of organic compounds tentatively identified in treated optimized pharmaceutical wastewater

Peak No.	Name of compound	$t_{R\ (min)}$	Molecular ion peak (m/z)	Fragment ion (m/z)
1	4-methoxybenzene-1,2-diol.	10.504	140	140
2	Naphthalene	13.431	128	128
3	2-Butenoic acid, 2 methyloxy-3methyl ester.	15.091	144	43
4	1-2Benzenedicarboxylic acid	21.946	148	142

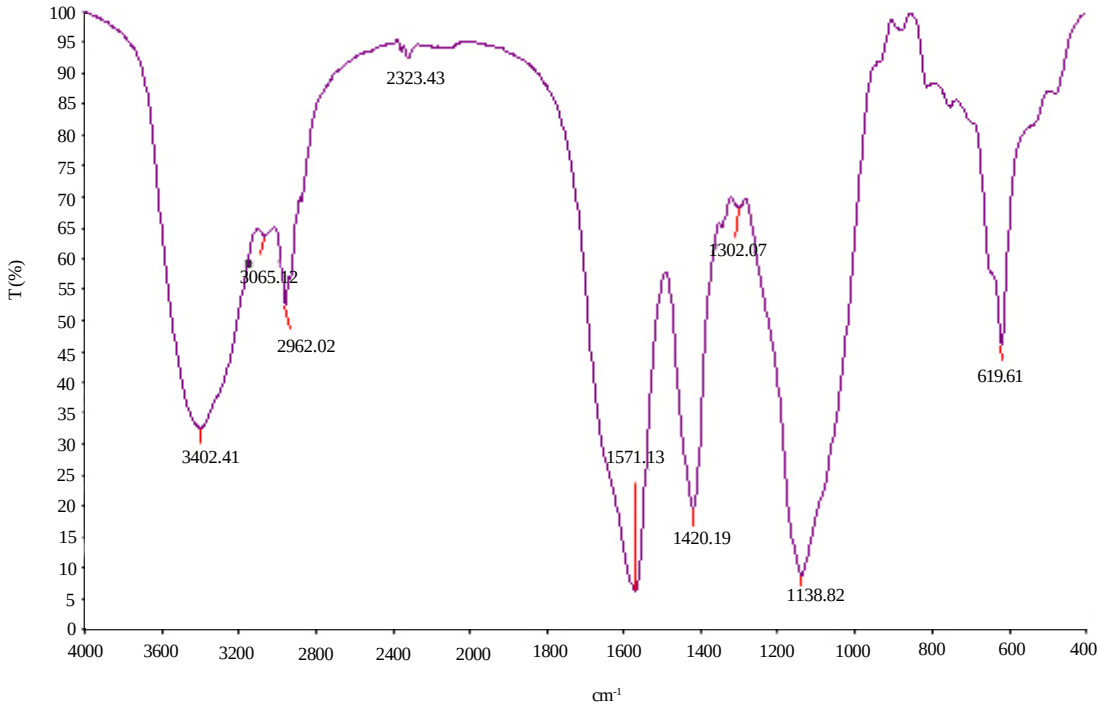

Fig. 2: GC-FTIR spectra of the pharmaceutical untreated wastewater (Fluka library supplied by Perkin-Elmer)

Effects of nitrogen source

The test water sample has a TN value of about 530 mg/L (about 0.05 % nitrogen by the weight of the BOD) which did not conform with the biological wastewater treatment of carbon to nitrogen ratio of 5 % nitrogen by the weight of the BOD (Kakabadse, 1979) bearing in mind the load of recalcitrant organic pollutants. Hence, the need for additional nutrients in the form of either $(NH_4)_2SO_4$ or $NaNO_3$. Fig. 3a shows that $(NH_4)_2SO_4$ produced the highest dry cell weight (592.3 mg/L) at 4000 mg/L against $NaNO_3$ with (318.2 mg/L) at the same concentration. Ammonium being the best nutrient was tried further at higher concentrations (Fig. 3b); it gave maximum growth of 672.0 mg/L DCW at 5000 mg/L while further increment retards the growth. Therefore, in order to treat this pharmaceutical wastewater, additional nutrients need to be added to the wastewater to stimulate increase in the growth of the microbes. This procedure is in accordance with the methods which have been commonly used in wastewater treatment (Kakabadse, 1979; Tchobanoglous *et al*., 2003). This experiment also confirmed that the isolate can utilize either form of nitrogen source (Kantachote *et al*., 2005).

Effects of yeast extract

The addition of yeast extract on ammonium supplemented wastewater further increased the cell growth of the PNSB from OD660 1.03 – 1.502 in the pharmaceutical wastewater test medium. It recorded 0.997g/L DCW at 1.0 g/L concentration. This cultivation was carried out over 96 h under microaerobic light conditions. High concentration had no significant effect (Fig.4). Although yeast extract contain many B vitamins, it may also act as a source of protein and its use depends on the economic evaluation of the process.

Effects of light intensity

In order to optimize the treatment conditions, the incident light intensity were varied between 500 to 8000 lumens. The best growth was observed at 6000lx (Fig. 5) with a COD reduction of about 75 % (initial COD value of 2500 to final value of 604 mg/L, respectively) and the cell growth was 820.5 mg/L DCW. While a temperature range of 20-30 ºC depending on the ambient temperature was maintained. This finding could be attributed to the fact that the PNSB thrives better in an intense light.

Optimization of wastewater treatment conditions

Fig. 6 shows the experiment carried out to infer the effective retention time for the treatment. The isolate grew up to 377.4mg/L in SW in 5 d and almost double the amount in SOW (688.3mg/L) at the same 5 d retention (Fig. 6a). Also Fig. 6b shows that the COD reduction was higher in SOW at 5d than SW (64.8 and 43.0 %, respectively), although at 7d, the COD and dry cell weight were slightly higher than at 5d, but 5d was

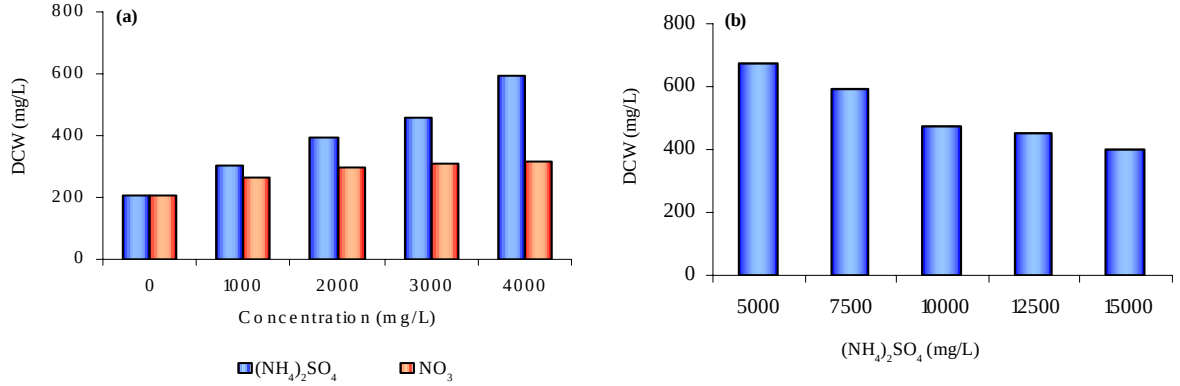

Fig. 3: Effect of nitrogen sources and their concentrations on the growth of the PNSB in pharmaceutical wastewater under microaerobic light conditions for 72 h; (a) Source of nitrogen; (b) Added $(NH_4)_2SO_4$ on bacterial growth

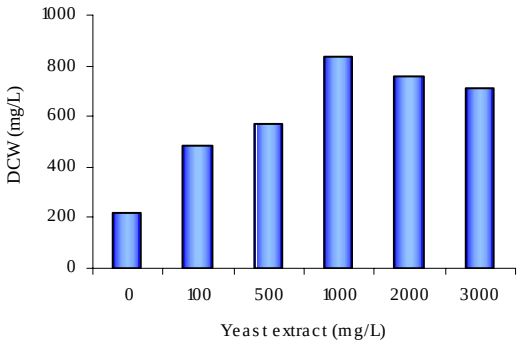

Fig. 4: Effect of added yeast extract and the concentration on the growth of PNSB in pharmaceutical wastewater under microaerobic condition for 72h with $(NH_4)_2SO_4$ = 5 g/L as base control

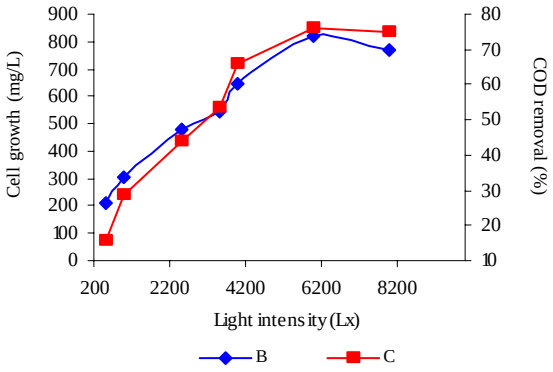

Fig. 5: Effect of light intensity variation on % COD removal and cell growth (mg/L). C = % COD removal, B = Cell growth

chosen as the optimal retention time for the treatment for economic reasons. The SW with lower COD reduction could be attributed to the inhibition of the cell activity by the recalcitrant pollutants in the wastewater (Table 1), while the sharp reduction of COD in the SOW with inoculums shows that the bacteria activity was increased with additional nutrient hence able to metabolize most of the pollutants faster before the onset of inhibition. There was no COD reduction or cell growth in SOW without inoculation which indicates that no mineralization of pollutants occurred by photo oxidation as COD is the amount of oxygen necessary to oxidize both the organic and inorganic matters to CO_2, H_2O and NH_3 (Bitton, 2005).

The conditions chosen for the treatment of the pharmaceutical wastewater were therefore as follows: supplemented wastewater was with 0.5 % ammonium sulphate and 0.1% yeast extract with a final pH of 6.6-7.0, incubation was without shaking at 20-30 ºC for 5 d with an incident light intensity of 6000 lx using fluorescent compact light also the non-supplemented wastewater. The choice of fluorescent compact lamp for illumination of the PBR was as a result that greater proportion of its power is converted to usable light while smaller proportion is converted to heat as against incandescent lamp which is heat driven and energy draining lamp. Although the initial cost of fluorescent lamp is higher than the incandescent lamp but fluorescent's longer life reduces lamp replacement cost. Typically a fluorescent lamp will last between 10 – 20 times as long as an equivalent incandescent lamp when operated several hours at a time. Therefore, the higher initial cost of a fluorescent lamp is usually more than compensated for by lower energy consumption over its life.

The growth kinetics of the PNSB in batch culture (data not shown), depict that the bacteria maintained speedy growth after 19 h of cultivation and reached saturation after 5d. From the result, the specific growth rate was calculated as 0.015 h^{-1} and 0.011 h^{-1} for SOW and SW, respectively. While the process productivity based on the five days optimal growth is 0.082 g/L/day with a biomass yield of 12.8 % for the optimized wastewater based on the chemical oxygen demand.

The protein content of the culture after treatment in both the supplemented and non-supplemented wastewater is well above the required protein content for it to be acceptable as a protein source for either fish feed or animal feed (Howard, 1987). Therefore, there is a great potential for the culture to be used as a SCP. However, the process productivity was low (0.082 g/L/day). Photosynthetic bacteria typically have lower process productivity than heterotrophic bacteria because the cell densities achieved are low (Kantachote *et al.*, 2005). As for the yield, normal heterotrophs growing aerobically could convert 50 % of organic carbon into biomass, whereas with anaerobic conditions only 5 % is converted into biomass (Speece, 1983). For this microaerobic condition the yield is about 12 % which is much favorable than complete anaerobic condition. Also, in this present study, the bacteria did not thrive well in a complete anaerobic light condition, for instance, after about 2weeks cultivation anaerobically, its optical density was (OD_{660} = 0.442 ± 0.0014). Whereas, at microaerobic light, it recorded greater optical density values in less than 2 weeks of

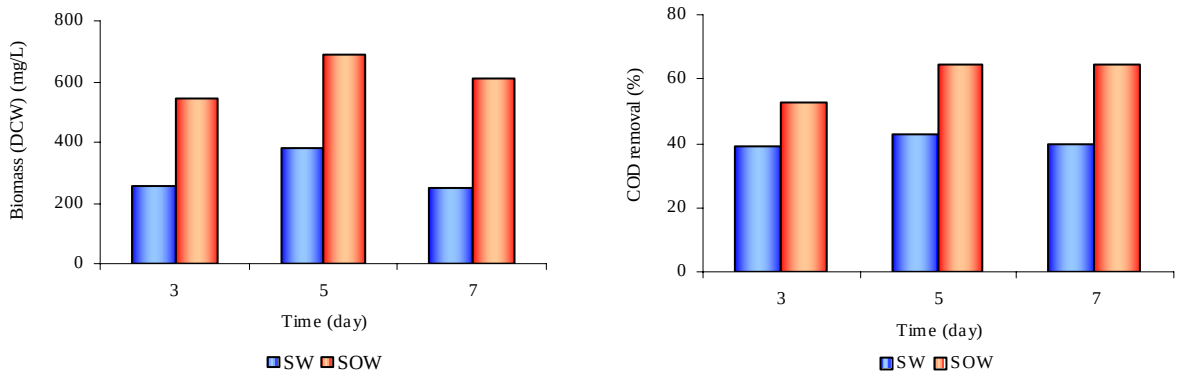

Fig. 6: Cell growth and COD removal as a function of time (at 3500lx, initial COD = 2500mg/L); (a) Biomass in mg/L DCW; (b) % COD Removal; SW = non-supplemented wastewater; SOW = supplemented wastewater; $(NH_4)_2SO_4$ =5 g/L and YE = 1 g/L)

cultivation (OD_{660} = 1.234 ± 0.0020). This suggested that the isolated PNSB could thrive better in microaerobic light than anaerobic light condition.

CONCLUSION

Better waste management will lead to other environmental benefits such as reduction of surface water and groundwater contamination and transformation of organic waste into high-quality manure. Therefore, the treatment procedure could be adopted by the chemical/pharmaceutical industries as interim measure in cubing their pollution problem particularly in most developing countries with abundant sunlight. Wild strain PNSB that is *Rhodobacter spheroides* z08 has proven to be effective in ameliorating hazardous pollutants found in pharmaceutical wastewater with over 80 % COD reduction and has the potential to improve the treatment process without any considerable increase in cost. It may also be harvested and find use as a SCP. However, further investigation must be carried out to ensure that the produced biomass is innocuous before its use as a SCP; otherwise, the best option is to use the biomass for energy production. Further work is needed to critically ascertain if the isolate is a toxic tolerant PNSB.

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