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Anoxygenic phototrophic bacteria and their biotechnological applications

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The anoxygenic phototrophic bacteria are, in general, anaerobic. They are prokaryotic and unique photosynthetic organism which lack photosystem II and thus carry out anoxygenic photosynthesis. The anoxygenic phototrophic bacteria are morphologically diverse and widely distributed in nature. Although anoxygenic phototrophic bacteria have been known for more than two centuries, their protential for various biotechnological applications has been recently recognized. The capability of anoxygenic phototrophic bacteria to degrade a number of inorganic compound (H_2S,thiosulphate) and organic compounds (fatty acids,alcohol, carbohydrates and even aromatics)make them suitable for exploitation in waste water treatment. In North Bihar, a number of sugar factories and one tannery factory is situated.Waste water from these factories contain both organic and inorganic matter and raw leather along with chemicals used in processing. such as ammonia, formic acid, sodium metasulphite and sodium sulphite. The isolate SS_4 had the best potential for the use in waste water tretment. A total 67 isolates of the purple non-sulphur photosynthetic bacteria (PNSB) were isolated from 9 smmples of waste water obtained from sugar-tannery factories. Optimum growth of SS_4 was obtained after supplementing the weste watter with 0.50% $(NH_4)_2 So_4$ and Img/L nicotinic acid. Using these optimum condition for growth SS_4 along with indigenous micro organism a reduction of 90% of both COD and BOD was achieved . Chemical analysis of cultures after treatment of the enriched waste water shows that the protein content of the pure SS_4 was 56.6% of the dry weight. Hence, single cell protein (SCP) may be possible biproduct of the treatment process.

Key words: Biochemical oxygen demand (BOD), Purple non sulphur photosynthetic bacteria (PNSB), Sugar factory effluents, Chemical oxygen demand (COD), Raw waste water (RWW),Optical density.

INTRODUCTION

Anoxygenic phototrophic bacteria,especially purple non sulphur photosynthetic bacteria (PNSB) are widely distributed in soil, water and waste water. Purple non sulphur photosynthetic bacteria (PNSB) are versatile organisms as they can grow as both photoautotrophic and photoheterotrophs under anaerobic light or micro aerobic-light conditions (Holt *et al.* 1994). They also can grow anaerobically in the dark using fermentation and many can grow aerobically in darkness using respiration. These PNSB can use various substrates as sources of carbon and energy with ammonium and / or nitrate as a source of nitrogen and may use sulphide or thiosulphate as an electron donor under photosynthetic conditions (Imhoff and Truppeer, 1989). Because of these properties, they have potential for treating various sources of waste water. In addition, single cell protein (SCP) may be by-product of waste water treatment and be used for animal feed (Pansano *et al.* 2002).

Sugar factories are established in entire north-Bihar and one Tannery factory is situated just at the bank of River Ganga at Mokamah (District-Patna of Bihar). Waste water from these factories contains both organic and inorganic matter that originated from natural standing crops and raw leather along with chemicals used in processing, such as ammonia, formic acid, sodium metabisulphite and sodium sulphite.

The effluents coming out from these factories either directly discharged to the tributary of Adhawara groups of river or it is being pretreated by

constructing lagoons or oxidation ponds. This treatment is low cost operation but produces hydrogen sulphide and the rotten egg odour is a major problem of the system.Many researchers have found that certain PNSB species from the genera *Rhodopseudomonas, Rhodobacter* and *Rhodospirillium* can eliminate the H_2S odour nuisance from the facultative ponds of waste stabilization ponds due to their ability to oxidise sulphide to sulphate using light during photolithoautotrophic growth (Tadesse *et al.,* 2003 & Kim *et al.,* 2004). The reddish-pink, deep brown with froth bloom of PNSB occurs occasionally in anaerobic or oxidation ponds of Tannery factory and Sugar factories.This means that some PNSB have a potential for use in the treatment of effluents discharged by above mentioned factories situated in Bihar. Therefore, the aims of this study are to screen for a strain of PNSB with this potential and to determine the optimum condition for this strain to be most effective in treating waste water hence it is a Biotechnological application of anoxygenic phototrophic bacteria.

MATERIALS AND METHODS

Waste water or effluents from Suger factories and Tannery factory at North and Central Bihar where a series of lagoon are used for the waste water system and water temperatures in these lagoons vary between 28-34^{0}C depending upon the sunlight in between the month of April-July. Waste water from the first lagoon was collected for this study because it caused an odorous (H_2S) nuisance. The properties of the waste water varied at each collecting time. However, the chemical oxygen demand level was between 4800 to 6800 mg/L and the total nitrogen level was between 18-28 mg/L. Volatile fatty acids (VFAs) were present with acetic acid at concentrations between 380 and 610 mg/L and the pH varied from 7.8 to 8. To determine above finding APHA, AWWA and WPCF(1998) was adopted. The collected waste water was centrifused at 5500 RPM for 15 min. followed by autoclaving 121^{0}C for 15min to achieve sterile conditions prior to use as the growth medium.

Microaerobic-light conditions were used for all experiments to make conditions as close as those of a natural system of waste water treatment. A total 9 waste sample (5 from Sugar factories and 4 from Tannery factory) were collected. 5ml. of each sample was inoculated into 5 ml of double strength of McKn broth for isolating PNSB and incubated at room temperature (28-32^{0}C) under anaerobic conditions. To achieve anaerobic-light condition,1 cm. sterile liquid paraffin was added to the top of the growth medium held in a test tube with a light intensity of 2000 lux generated by fluorescent light for between 48 to 96 hrs. Purification of single colony was achieved by successive re-streaking on McKn medium containning 1.5% agar and incubated in same conditions. McKn medium supplemented with an optimal concentration (0.5%) of $(NH_4)_2 SO_4$ was used as base control.

RESULTS AND DISCUSSION

With anaerobic light condition and McKn medium (water sample medium = 1:1), with malate is a sole source of carbon, 23 isolates of PNSB were readily isolated from 9 samples collected from lagoon of these factories. 17 isolates that grew with microaerobic-light condition after 72 hrs incubation period, were selected for further screening due to their growth exceeding an $OD_{660 \text{ of } 0.10}$ in sterile Tannery factory effluent (waste water) medium without the addition of any nutrients. After 72 hrs incubation many isolates of PNSB grew well an $OD_{660 > 0.80}$ in a medium supplemented with 0.1% YE (yeast extract) either in sterile waste water (SW+0.10%YE) or in raw (non autoclaved) waste water (RW+0.10% YE). Without the supplement of YE both in the SW and the RW, only isolates SS_1 and SS_4 gave growth > 0.30 $OD_{600,}$ However, only the isolates SS_4 was selected for further studies as it was one isolate that also produced grown > 0.30 OD_{600} in the raw unsupplemented waste water in competition with the indigenous organisms.

Table 1 : Effects of supplementing the sugar factory waste water medium with vitamins or YE on growth of the isolate SS_4 under microscopic light conditions at 30^{0}C

Supplementation	Concentration (mg/L)	Growth (COD 660 nm)
No supplement (Control)	0	1.55
Biotin	0.01	1.18
Nicotinic acid	1	1.79
P-amino benzoic acid	1	1.63
Thiamine hydrochloride	1	1.07
Yeast extract	1000	2.00
Basal medium	McKn (broth)	1.17

Supplements of nitrogen to SW in the form of ei-

ther $(NH_4)_2 SO_4$ or $NaNo_3$ produced better growth of the isolates SS_4 under microaerobic light conditions that SW with no added nitrogen source. More growth was obtained with ammonium ion with nitrate ion, and the optimum ion concentration was 0.50% (5g/L) with higher levels retarding growth. Over 72 hrs of cultivation with microaerobic-light conditions the best growth of isolates SS_4 was observed in SW with YE added at I g/L ($OD_{600\text{-}2.00}$). Addition of nicotinic acid to concentration of I mg/L increased the OD_{600} at the end of cultivation to 1.79 from 1.55 for the control, but higher concentration had no additional effect.Supplementing with P-aminobenzoic acid to a concentration of I mg/L increased the $OD_{600\text{ to }1.63}$. In contrast, the addition of biotin (0.01 mg/L) or thiamin hydrocholoride (1 mg/L) or all vitamins together reduced growth.

The growing bacteria produced significant reduction of BOD and COD. The small loss of BOD (5%) with no COD reduction in SOW indicates that abiotic degradation occurred and this was possibly due to photo-oxidation of some non-organic material also to contribute the BOD. This also caused some increase in turbidity and pH. The absence of COD reduction in SOW indicates that no mineralization of organic matter occured by photo-oxidation as COD in the amount of oxygen necessary to oxidise organic matters to CO_2,H_2O and NH_3 (Bitton, 1994).In the case of ROW there was a higher reduction of BOD (70%) than of COD (54%).

The protein content of PNSB strain SS_4 (65.2%) was similar to that of *Rhodocyclus gelatinosus* R_1 (67.7%) growth with poultry- slaughter house waste water under anaerobic light conditions (Ponasano *et al.*, 2003). Isolates SS_4 was therefore great potential for use of SCP (Single cell protein). However, the process productivity of the isolate SS_4 was low (0.10 g/L day) but much better (2.4 g/L days) in the presence of indigenous microbes. Photosynthetic bacteria typically have lower process productivity than heterotrophic bacteria because the cell densities achieved are low.

In conclusion PNSB strain SS_4 when added to a system treating waste water from Tannery industry and Sugar factories has the potential to improve the treatment process without any considerable increase in cost and may not be harvestd and further tests for absence of toxicity find use as SCP (Single cell protein).

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