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Full Length Research Paper

Microaerobic biodegradation of high organic load wastewater by phototrophic bacteria

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High organic strength artificial simulated brewery wastewater (10,000 mgl^{-1} COD) was used in a batch photo-bioreactor to study the effectiveness of new strain phototrophic bacteria in bioremediation of organic contaminated wastewater. In this work, effects of additions of three individual micronutrients, that is, ferrous, molybdenum and magnesium, and a mixture of micronutrient with urea on the performance of *Rhodobacter sphaeroides* Z08 in biodegradation of high organic load wastewater under varied light intensities were investigated. Maximum organic contaminates reduction were 42.3% for ferrous, 36.1% for molybdenum, and 32.3% for magnesium with the corresponding biomass of 989.7, 978.8 and 888.6 mgl^{-1}, respectively. Urea addition increased the wastewater biodegradation potential with the resultant change in carbon to nitrogen ratio (C:N) from initial 200:1 to 200:5. Binary supplement of 26 mgl^{-1} ferrous and 200 mgl^{-1} urea to the wastewater readily enhanced the bacteria activity leading to organic contaminates reduction of 67.6%, while optimum pollutants reduction was achieved when binary supplement of urea and ferrous was made under intense radiation of 4000 lumens yielding nearly 80, 48, 90 and 67% reductions of chemical oxygen demand, total phosphorus, total organic carbon and total nitrogen, respectively. In addition, analyses of the resultant biomass and the purified wastewater indicated that major inorganic constituents of the wastewater were assimilated.

Key words: Biodegradation, environmental pollution, phototrophic bacteria, micronutrients, macronutrient, wastewater treatment, artificial light intensity.

INTRODUCTION

High strength organic wastewaters constitute nuisance cum waste of valuable potential resources to the environment. Large flow of these wastewaters are been generated by various plants, especially the food industries, and need treatment before been discharged into the environment in other to meet the stringent regulations that are being imposed by various government agencies (Briggs et al., 2004). In recent times, organic contaminated waste waters are being purified by photosynthetic bacteria (PSB) that can purify heavily polluted water when exposed to sunlight (Okubo et al., 2006). As a result of their unique advantages of solar radiation as energy source, phototrophic bacteria are able to take advantage of organic matter to synthesize bacterial protein; therefore, a number of non-toxic wastewater could be purified by phototrophic bacteria while the cell protein could be recovered (Choorit et al., 2002).

Dating from the time Kobayashi and Tchan (1973) reported the application of PSB in wastewater treatment, many researchers had explored the potentialities of PSB in wastewater remediation and resource recovery (Honda et al., 2006; Do et al., 2003; Myung et al., 2004; Ding, 2008). The use of PSB for wastewater treatment has been favored by many researchers because they are metabolically versatile, anaerobic photoautotrophic and photoheterotrophic in the light and microaerobic-light condition (Holt et al., 1994). They can also grow anaerobically

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Abbreviations: PSB, Photosynthetic bacteria; **SW,** simulated brewery wastewater; **ICP,** inductive coupled plasma; **GC-FID,** gas chromatography flame ionization detector; **COD,** chemical oxygen demand; **TOC,** total organic carbon; **VFAs,** volatile fatty acids; **TN,** total nitrogen; **TP,** total phosphorous; **DCW,** dry cell weight; **TKN,** total khjeldal nitrogen.

Table 1. Composition of modified sistrom minimal medium.

Nutrients (gl^{-1})	Concentration (gl^{-1})	Mineral and Vitamin	Concentration (gl^{-1})
$(NH_4)_2SO_4$	1.0	$MnCl_2.4H_2O$	0.003
D, L malate	4.0	$CoSO_4.7H_2O$	0.02
$MgSO_4.7H_2O_2$	0.12	$ZnSO_4.7H_2O$	0.01
$CaCl_2.2H_2O_2$	0.076	H_3BO_3	0.03
EDTA	0.02	$NaMoO_4$. 2H2O	0.003
K_2HPO_4	0.39	$CuSO_4$. $5H_2O$	0.001
KH_2PO_4	0.59	Yeast extract	0.10
$Fe(S0_4)_3.7H_2O_2$	0.0065		

in the dark using fermentation and many are aerobically chemo-heterotrophic in the dark (Lorrungruang et al., 2006). Conventionally, high organic load wastewater treatments lie mainly in anaerobic treatment process that yields effluent with high energy rich molecules. Recently, microearobic treatment process has been encouraged to be explored for some reasons. As a transition phases between aerobic and anaerobic environment does not require high energy input and its resultant effluent is not ridden with high energy rich molecules, PSB easily degrade these energy rich molecules and convert them into cellular matter (Haun, 2005; Matsunaga et al., 2000; Libing et al., 2006). Our effort here is to develop a low cost bio-treatment process for high organic load waste-waters, and microaerobic environment stands the best option because of it economic savings. Therefore, in this study, we specify optimum strategies for the application of a new strain of PSB in organic saturated wastewater under limited oxygen environment. Hence, we proposed microaerobic bioremediation of artificial laboratory simulated brewery wastewater (SW) by a new PSB named *Rhodobacter spheroides* Z08. The effect of metal cations, oxyanion, macronutrient and light intensity as well as chemical analyses of the accumulated biomass from the remediation process was used to deduce the potential application of the biomass as a useful by-product. In addition, inductive coupled plasma (ICP) and gas chro-matography flame ionization detector (GC-FID) were employed to assay the SW for inorganic nutrients, some organic acids (acetic, propionic, butyric and valeric) and alcohol concentrations. This is a detailed study on characterization and bioremediation of binary enriched organic wastewater with macro and micro nutrients under microaerobic environment.

MATERIALS AND METHODS

Inoculums preparation

A new strain of *R. spheroides* named Z08 was gotten from the culture bank of State key Lab of Urban Water Resource and Environment (Harbin, China). Inoculums were prepared by growing the cells in a modified Sistrom's minimal malate (RCVBN) medium (pH 6.8) microaerobically in a reciprocating thermostatic shaker (unitron, Infors AG, Germany) at 200 rpm for 48 h within a temperature range of 28 - 30°C. Nitrogen source was ammonium sulfate (0.99 g/l) and carbon source was D-malate (4.02 g/l). Detailed recipe on the growth media is provided on Table 1. Sterilization of the medium was accomplished by autoclaving at 121°C for 15 min; inoculation into the medium was as 10% v/v inoculums from a culture growing in the modified RCVBN medium. For use as inoculums, cell suspension was adjusted to the desired optical density (0.5) at 660 nm using sterile distilled water as diluents. Sterile distilled water was also used as the blank.

Wastewater preparation

High organic strength wastewater was simulated in the laboratory by dilution of free beer to cover wide range of pollutants strength as could be generated by various organic wastewater generating industries since organic industrial wastewater strength is dependent on the raw material constituents (Bitton, 2005). The organic pollutants were represented by chemical oxygen demand (COD) and various strength of the wastewater COD were simulated ranging from 1050 – 10,000 mgl^{-1} COD. SW was characterized based on the followings; COD, total nitrogen (TN), total phosphorous (TP), ammonia-nitrogen (NH_3-N), total organic carbon (TOC) and volatile fatty acids (VFAs).

The simulated artificial wastewater COD is approximately 10,000 mgl^{-1} COD, total phosphorous ~260 mgl^{-1}, total nitrogen ~50 mgl^{-1}, for inorganic nutrients, iron was lower than the concentration in the original Sistrom minimal (RVCBN) medium, while molybdenum was completely unavailable and magnesium retained its usual higher concentration in the simulated wastewater as in the RCVBN medium.

Optimization of wastewater composition and treatment conditions

SW with pollutants strength of 3600 mgl^{-1} COD was used to incubate the phototrophic bacteria microaerobically in the light (approximately 2000 lx), the COD reduction was monitored for at least 7 days, based on the results, an order of reaction curve was drawn to elucidate the reaction pattern of the SW moieties. Effects of metal cations (Fe^{2+} and Mg^{2+}), oxyanion (MoO_4^{2-}), and macro-nutrient (NH_2-CO-NH_2) supplements were examined consecutively on the treatment efficiency with SW strength of 10,000 mgl^{-1} COD. PSB are light dependent organisms with a unique advantage of solar radiation as energy source, average incident light intensity on the bioreactor was also optimized using various lumens (lx) 500, 1000, 2000, 3000, 4000 and 5000, respectively. The source light was a 40 W compact fluorescent lamp mounted on one side of the bioreactor. Variations were obtained by adjusting the distance between light source and the bioreactor.

Table 2. Physicochemical composition of the SW (parameters in mgl^{-1}, except pH).

Parameters	Wastewater
COD	10,000±0.05
TP	360.5±0.02
TN	51±0.02
pH@25°C	4.7±0.03
NH3-N	0.894±0.002
Mg	5.438±0.01
Mn	0.028±0.003
Mo	ND
Iron	0.024±0.003
Ethanol	4335±0.23
Acetic acid	-
Propionic	-
Butyric	-
Valeric	-

ND = Not detected.

Analytical methods

Organic acids (valeric, butyric, propionic and acetic acids) and alcoholic content of the SW influent and effluent were measured using GC-FID as described by Shi and Yu (2006). Incident light intensity on the bioreactor was measured with a TES-1330A digital light meter. The bacterial cell concentration was determined by optical density at a wavelength of 660 nm (OD_{660}) with a UV-VIS spectrophotometer (Shimadzu UV-120), the dry cell weight (DCW) was by conversion of OD_{660} values to DCW via a proper calibration curve (where1.0 OD_{660} approximately equals 689 mg dry cell/l.). COD, TP, NH_3-N and pH were measured according to standard methods (Alpha, 1992). TOC and TN were determined by a total organic carbon analyzer coupled with total nitrogen measuring unit (Shimadzu). Trace metals were measured using a Perkin Elmer Optima 5300DV ICP aided by a WTW microwave digester. Crude protein content was determined by multiplication of total khjeldal nitrogen (TKN) content with a conversion factor of 6.26 (Roulston et al., 2000). TKN content was determined using micro khjeldal apparatus. Prior to the analysis, the biomass was freeze dried at 25°C and 760 torr for 6 h.

Batch photo-bioreactor

The experiments on the bioremediation kinetics of the SW by the new strain *R. spheroides* Z08 were conducted in a transparent (40 x 20 x 20 cm deep) glass vessel that was tapered at the edge with a muslin cloth and cellophane, the total volume of the vessel was 2 L, with a working volume of 500 ml. The experimental conditions were microaerobic environment at 20 - 30°C, and a pH of 6.0 - 6.5. Illumination was with a 40 W compact fluorescent lamp on one side of the bioreactor at the desired incident light intensity. Microaerobic condition was achieved as described in our previous work (Madukasi et al., 2010). Typical experiment was 500 ml SW with the supplement(s) dissolved in it, pH was adjusted, and mounted on a magnetic stirrer at a moderate speed. To initiate an experiment, inoculation into the SW was with 20% inoculums of 0.5 optical densities at a wavelength of 660 nm. For the biodegradation kinetics study, at fixed intervals of 12 - 72 h or more, 10 – 20 ml aliquot sample was withdrawn, centrifuged (9000 rpm, 15 min) and analyzed. The photobioreactor was non-autoclaved; prior to the experiment initiation, the bioreactor and its content was sterilized by UV radiation. Deoxygenation was done by placing the entire reactor in the dark for at least 1 h, no flushing with either argon or N_2 gas was done, rather 1.0 gl^{-1} ascorbic acid ($C_6H_5O_6$) was added into the bioreactor in most cases to lower the oxygen level as $C_6H_5O_6$ is a chemical reducer with a hydrogen potential of +0.08 V, making it capable of reducing such compounds as molecular oxygen and nitrate (Padh, 1990).

RESULTS AND DISCUSSION

Wastewater

The analyses in Table 2 show that the SW is high in organic pollutants, with COD, TN and TP values of approximately 10,000, 50, and 260 mgl^{-1}, respectively. The ratio of TN to TP is approximately 0.2; this indicates that the SW has a nitrogen limitation. Also some of the essential elements are low such as Fe (0.025 mgl^{-1}) and Mo is below detectable limit. The VFA's were undetectable in the raw wastewater with alcoholic content of approximately 4000 mgl^{-1}. These variations in the SW composition necessitated the need to identify the reaction order which is shown to be first order (Figure 1) depicting that the reaction is independent of the contaminants concentrations. Based on this finding, subsequent experiments were conducted with SW strength of 10,000 mgl^{-1} COD. *R. sphaeroides* was difficult to reproduce in a complete anaerobic light laboratory condition but thrived better in a microaerobic light as shown in Table 3.

Therefore, both the inoculums production and culture utilization for wastewater treatment were carried out micro-aerobically in the light under mesophilic temperature range (20 - 35°C) below which the bacteria did not bloom. From Table 3, it could be concluded that the phototrophic bacterium is a microaerophilic PSB which grows at reduced concentration of molecular oxygen basically by respiratory energy conversion at low oxygen partial pressure. An indication that too much oxygen is unsuitable was as a result of the fact that phototrophic bacteria grown in strict aerobic condition in a 40 x 20 x 20cm vessel with complete mixing by air circulation decreased in number in both dark and in light conditions as shown in Table 3.

The effects of oxyanion and cations

The effects of oxyanion and cations (Mo^{2-}, Mg^{2+} and Fe^{2+}) supplements on the treatment efficiency were investigated by the addition of corresponding salts of Mo ($Na_2MoO_4.2H_2O$), Mg ($MgSO_4.2H_2O$) and Fe ($FeSO_4.7H_2O$), respectively. In the case of Fe^{2+} addition, it was found to be beneficial up to 26mgl^{-1} in both cell growth and pollutants reduction either as a single supplement or in combination with other supplements.

Figure 2 shows that addition of Fe^{2+} up to 26mgl^{-1} supported and sustained a stable growth for a longer period

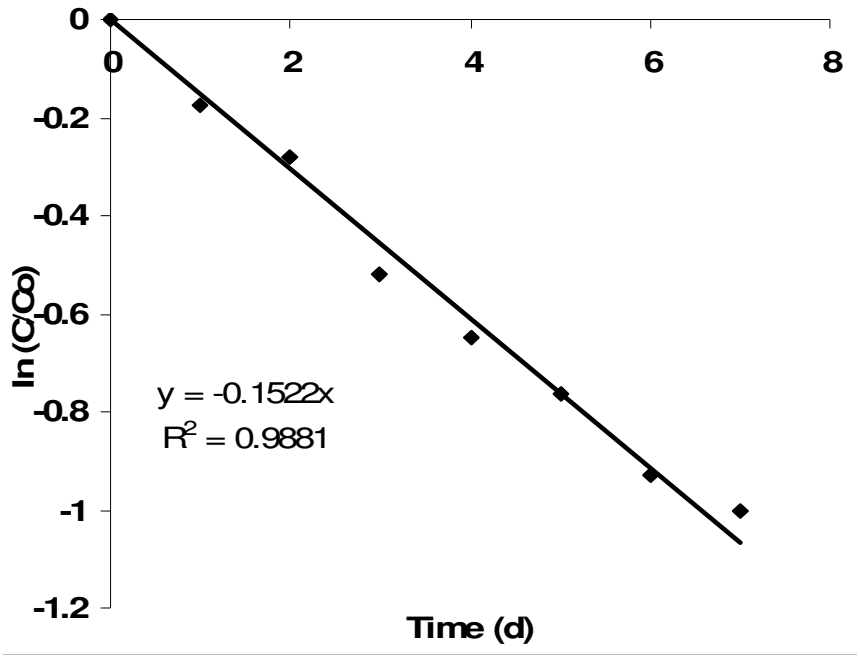

Figure 1. Characteristic reaction order of the SW (SW initial COD = 3600 mgl^{-1}).

Table 3. Phototrophic bacteria growth in simulated brewery wastewater under different conditions (mgl^{-1}DCW).

Incubation	Initial	After 3 days	5 days	7 days	10 days
Light					
Aerobic	136.2	119.4	128.3	132.0	149.3
Microaerobic	136.2	581.4	867.1	1070.3	1647.8
Anaerobic	136.2	221.0	265.2	293.0	302.0
Dark					
Aerobic	136.2	116.3	121.2	128.0	146.4
Anaerobic	136.2	102.0	123.2	136.3	148.6

Initial SW COD = 10,000 mgl^{-1}; 40W FCL (1910 - 2022 lx), FCL = fluorescent compact lamp.

(over 7days) without decline with a steep increase in COD percentage removal. In contrast, its increase up to 50 mgl^{-1}, increased cell growth and contaminants reduction up to 5 days hydraulic retention (HRT) and declined steeply afterwards with the formation of a green-like rust phase, and consequently pollutants conversion ability decreased as well as the cell growth. Green rust as described by Christiansen et al. (2009) is a family of Fe (II), Fe (III) layered hydroxide that is present in environment close to the Fe (II)/Fe (III) transition zone. Its high reactivity makes it unnoticed in most cases at high oxygen concentration as it transforms readily to Fe (III) phase. This effect could be one of the possible causes of attainment of a stationary phase which stalls increase in the cell growth and subsequent attainment of dead phase. Also, with convectional oxygen circulation in the bioreactor, green rust readily transforms to Fe (III) phase which is less soluble and non-available for bacteria assimilation. This could lead to toxicity accumulation with resultant attainment of dead phase by the organisms. Iron is an essential nutrient for all living organisms. It is a cation that exists in the ferrous (Fe^{2+}) and ferric (Fe^{3+}) states. In biomolecules, its redox potential (Fe^{2+}/Fe^{3+}) spans a range from +300 to -500 mV, which makes it well suited for participation in electron transfer reactions (Bacquevort et al., 2007). Table 3 confirms that Fe^{2+} addition played double role in this experiment, firstly, as an essential bacterial cellular component that aids in cell respiration and electron transfer, hence the better growth of the cell as indicated by the DCW when compared with the cell growth upon addition of the other two inorganic nutrient supplements. Secondly, ferrous could have utilized its ability as a chemical reducer and supplied electrons that aided in reductive transformation of some

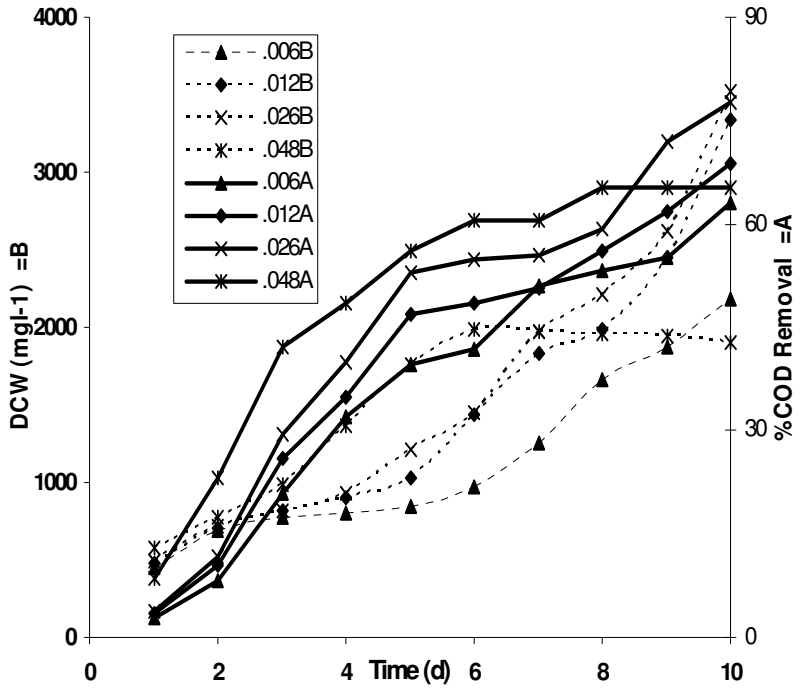

Figure 2. Effects of ferrous concentrations on the cell growth and COD% removal, at approximately 2000 lx incident light intensity. A = COD% removal and B = cell growth (DCW mgl^{-1}).

organic contaminants, hence the greater COD percentage removal when compare with the other inorganic supplements. The role that ferrous iron plays as an electron source for the reductive transformation of organic contaminants has been previously reported (Hong and Gu, 2009; Pauldyal et al., 2007). Iron is an important co-factor of many enzyme complexes and component of bacterial cellular components such as ferroprotoporphyrin (heme) groups which are essential moieties of many enzymes involved in bacterial respiration, electron transport and peroxide reduction, also iron containing non heme is required for DNA synthesis, protection from superoxide and amino acid biosynthesis (Pauldyal et al., 2007). But its addition in a system is not endless, in a biological system, bacterial iron uptake is in response to the cytoplasmic Fe^{2+} concentration which has a limit, otherwise iron toxicity will set and this could lead to attainment of early stationary phase and consequently, dead phase. Yegani et al. (2005) found that increase in Fe^{2+} from 12 to 48 mgl^{-1}sustained stable growth of *Rhodobacter capsulatus* B-100, a phototrophic bacterium.

For molybdenum (Mo), its addition up to 200 mgl^{-1} as a single supplement enhanced and sustained cell growth and contaminants reduction for over 7 days cultivation without attaining stationary phase, whereas, its addition in combination with other inorganic nutrients (Table 4) yielded no significant effect on both the COD reduction and cell growth when compared with its sole addition. These effects could be explained that although Fe^{2+} and Mo^{2-} are inevitable part of cellular component and are important co-factors of enzyme complexes, catalyzing basic cell functions such as electron transport, redox reactions and energy metabolism (Pauldyal et al., 2007), and the inability to impact positively on the pollutant reduction or cell growth when binary addition of Mo^{2-} and Fe^{2+} were made, indicate that the two ions have no synergistic effect in biological system, and both ions limitation or overload delay cell growth and could eventually cause cell death. More importantly, Fe^{2+} is a chemical reducer, while Mo^{2-} is an oxidizer, the presence of the two elements in higher concentration in the system could have nullified their redox effect on the contaminates, hence there were no better increase in either cell growth or percentage pollutants reduction as recorded by the DCW and COD% removal in Table 4. Also Wang et al. (2009) and Borch et al. (2007) had previously reported in their works that many oxyanions interact strongly with iron via surface complexation, thereby could affect iron reducibility, stability and mineralization pathway. In this study, we reported that TP concentration of the SW is relatively high, approximately 360 mgl^{-1}. Addition of 200mgl^{-1} Mo^{2-} in the reactor could have resulted in a negative impact on Fe^{2+} ability to improve the cell growth and activity as a result of complexation effect with the oxyanions (phosphate and molybdenum) ions present in the bioreactor.

Magnesium is the most abundant divalent cation in living cells and often functions in conjunction with the energy

Table 4. Process performance of wastewater treatment system based on sole and binary additions of the inorganic nutrients (Fe, Mo and Mg (mgl^{-1}).

HRT (d)	Fe (26)		Mo (200)		Mg (400)	
	DCW	COD	DCW	COD	DCW	COD
1	490.0	3.8	580.0	5.2	560.0	4.7
2	720.5	15.6	682.5	13.8	628.0	10.2
3	899.5	36.7	978.8	36.1	888.6	32.3
4	956.6	41.8	990.0	42.5	912.0	38.6
5	1209.0	53.0	1170.0	52.5	1177.0	52.8
6	1453.0	54.7	1345.0	53.4	1394.0	53.5
7	1776.0	58.6	1701.0	56.3	1887.0	55.2
	Mo (200) + Fe (26)		Mg (400) + Fe (26)		Mo (200) + Mg (400)	
	DCW	COD	DCW	COD	DCW	COD
1	367.5	21.3	422.0	26.4	404.0	11.9
2	402.3	23.8	726.2	29.3	512.0	18.5
3	497.4	26.2	872.6	39.7	587.0	21.7
4	593.7	30.6	897.4	42.6	626.3	28.5
5	617.4	31.9	1266.8	54.6	696.4	29.6
6	602.7	31.9	1509.0	56.8	729.5	30.9
7	596.4	40.2	1987.3	61.3	746.3	36.4

Cultivation was at approximately 2000 lx; initial SW COD = 10,000 mgl^{-1}.

Table 5. Micronutrient of simulated wastewater before and after nutrient addition and the biomass (mgl^{-1}).

Components	Before	After (26 mgl^{-1} Fe + Mg 400 mgl^{-1})	Biomass
Fe2+	0.025±0.000	1.27±0.130	3.851±0.2
Mg2+	6.291±0.000	15.92±0.337	15.28±0.3
Mn2+	0.034±0.000	0.314±0.0007	0.173±0.001
Mo2+	-0.003±0.000	0.078±0.003	0.05±0.001

rich chemical adenosine triphosphate (ATP) in many enzymatic reactions, increase in its addition (i.e., more than the quantities in either the cultivation medium or the SW test medium) up to ~400 mgl^{-1} either as a single supplement or in combination with othersupplements also sustained growth and pollutants reduction. Experiments revealed that binary addition of Fe^{2+} + Mg^{2+} impacted positively on the reduction rate of the pollutants as recorded by the COD% removal (Table 4) more than the binary addition of Fe^{2+} + Mo^{2-} and Mg^{2+} + Mo^{2-}, respectively. Again this shows that Fe^{2+} and Mg^{2+} has a synergistic effect in biological system, the two ions being cations could have followed the principles of attraction.

Analyses of both treated SW and the biomass (Table 5) shows that major inorganic constituents of the wastewater were assimilated resulting in uptake of minerals, also the biomass constituents are non lethal depicting its potential application as a supplement for soil conditioner, although its crude protein content is relatively low (Myung et al., 2004).

The effects of macronutrient addition

The efficiency of the treatment processes was also investigated by supplementing the SW with urea (NH_2-CO-NH_2) as indicated in Figures 3a and b. It is shown elsewhere that sole addition of urea in the SW increased both the cell growth and pollutants reduction by 987.5 mgl^{-1} and 58.3% when added 300 and 927.6mgl^{-1}, and 51.3% when 200mgl^{-1} was added after 3 days HRT (result of an initial experiment, data not shown). Urea is a high protein compound and may result in excess residual nitrogen after treatment which could lead to eutrophication upon discharge of the treated wastewater. Based on this fact, subsequent utilization of urea was as 200 mgl^{-1} supplement. Addition of urea in combination with Fe^{2+} yielded better impact on both the pollutants reduction and cell growth, than sole urea addition or the SW without any addition which served as control. Best impact was the addition of 200 mgl^{-1} urea with 50 mgl^{-1} ferrous (which resulted to 75.8% COD reduction and 1,875mgl^{-1}

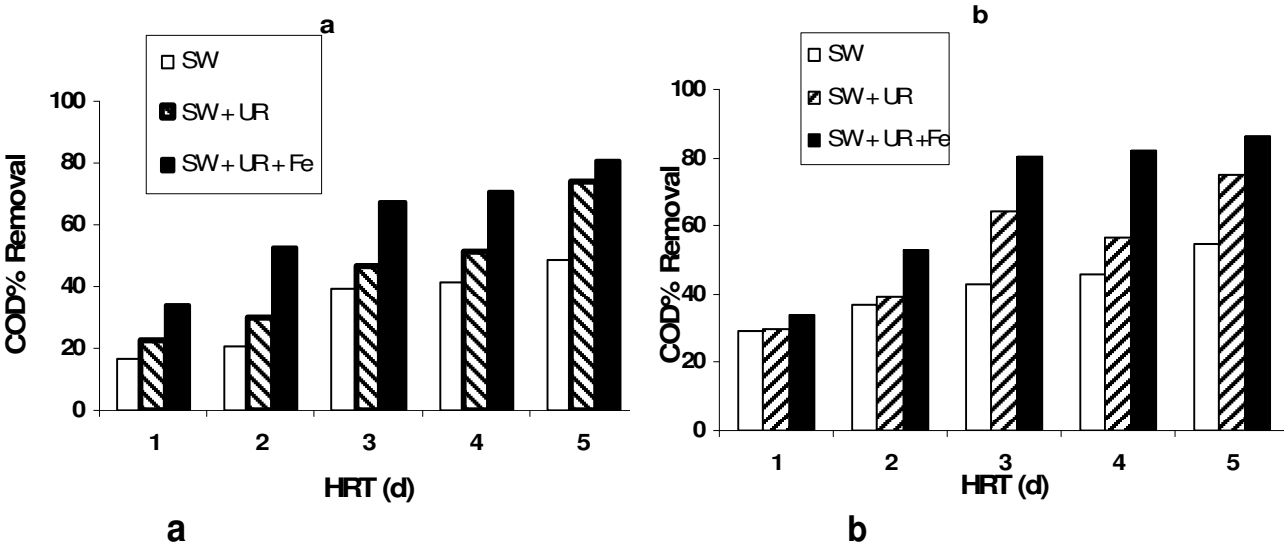

Figure 3. Effect of sole urea addition (SW + UR (200 mgl^{-1})) and urea plus ferrous (SW + UR + Fe (26 mgl^{-1})) on COD% removal at incident light intensity approximately 2000 lx (a) and 4000 lx (b). SW = simulated wastewater (control), UR = urea.

Table 6. Effects of incident light variation on the process performance at 3 days HRT (parameters = %).

Lumen (lx)	SW only			SW + Fe			SW + UR			SW + UR +Fe		
	COD	TP	TOC	COD	TP	TOC	COD	TP	TOC	COD	TP	TOC
500	24.0	8.2	36.4	31.6	10.2	43.0	37.0	14.0	48.3	32.0	39.0	46.0
1000	27.0	9.7	42.0	36.0	11.3	48.0	42.4	16.3	54.0	42.6	42.0	62.0
3000	34.4	10.7	46.0	48.0	16.4	59.3	57.0	21.6	67.5	64.3	46.0	78.0
4000	42.6	12.6	53.6	56.0	22.8	66.5	64.3	28.0	78.0	80.6	48.0	94.3
5000	43.8	13.2	54.0	58.4	23.6	69.4	69.0	29.3	81.0	84.2	49.2	96.4

UR = 200 mgl^{-1}; Fe^{2+} = 24 mgl^{-1}.

DCW) but for the same reason of the cells attaining early dead phase, subsequent utilization of urea and ferrous were 200 and 26 mgl^{-1} which yielded 67.6% COD reduction at 3 days HRT (Figure 3a). It has been reported that in Fe-replete areas, binary addition of macronutrient (glucose) plus Fe enhanced bacterial production and growth rates significantly more than single addition of macronutrient (Kirchman et al., 2000).

Effects of binary nutrients supplement and light intensity variation

Phototrophic bacteria are light dependent organisms; thus, effects of light variation were studied and it was shown that increase in the incident light intensity without nutrient addition had no direct impact on the process, but addition of nutrients improved the process performance significantly. For instance, increasing the incident light from 2000 to 4000 lx treating the SW without any supplement recorded 42.8, 12.6 and 53.6% reductions of COD, TP and TOC. Treating SW + Fe^{2+} recorded 56.0, 22.8 and 66.5% reductions while treating SW + UR recorded 64.3, 28.0 and 78.0% reductions, respectively. Below 2000 lx (500 and 1000 lx), COD% reductions were 24.0 and 27.0 for the SW without any supplement, 32.0 and 43.0% for SW binary supplemented with UR + Fe^{2+}. The best contaminants reduction was when the SW was binary supplemented with Fe^{2+} + UR (26.0 and 200.0 mgl^{-1}) at 4000 lx , which resulted in increase of the biodegradation rate as stipulated by COD, TP and TOC reductions of 80.6, 48.0 and 94.3%, respectively, detail result on this analyses is shown in Table 6. These findings suggest that the activities of this phototrophic bacteria specie depends strictly on nutrients availability since there is no direct correlation between incident light intensity variations and the pollutants reduction without nutrients supplement. Although the cells multiply readily both in supplemented and non supplemented wastewaters (data not shown), the activity was not improved without nutrients addition as indicated by the contaminants reductions. It has been reported that light is not a growth limiting factor for a phototrophic bacteria, Zhang et al. (2007) and Yegani et al. (2005) found out that phototrophic

Table 7. Volatile fatty acids and ethanol in treated and untreated SW.

VFA and ethanol mgl^{-1}	Untreated SW	Non-supplemented SW	SW + Fe + UR
Acetic acid	-	64.4	6.80
Propionic acid	-	6.0	0.58
Butyric acid	-	64.1	3.04
Valeric acid	-	35.9	2.22
Ethanol	4261.7	570.5	1.03

bacterium thrived best at illumination of above 1000 lx. Furthermore, in this present study, the phototrophic bacterial strain thrived well in the SW enriched with binary addition of Fe^{2+} + UR as stipulated in Figure 3a, more than sole addition of the other supplements at intensity radiation of approximately 2000 lx. The choice of light intensity supplement lies in the fact that the self shading effect that causes decrease in light penetration as the bacterial mass increases in the reactor predominates at incident light below 1000 lx (Chae et al., 2006) also artificial light supplement takes care of the dark and low light intensity period (night and day cycle).

Gas chromatography analysis

The GC analyses of both supplemented and non-supplemented treated SW and the untreated SW is summarized in Table 7. The absence of VFAs such as acetic, propionic, butyric and valeric acids in the untreated SW is an indication that the initial SW contains mainly insoluble particles or macromolecules such as starch, which is the reverse after treatment of both supplemented and non-supplemented SW. Again, this could explain that under microaerobic environment, phototrophic bacteria utilizes dual metabolic pathways, firstly, there is the photosynthetic energy conversion at the onset of the experiment when the oxygen level is very low, utilizing partial fermentative pathway with the resultant high energy rich molecules as the products. Secondly, photobacteria grows mainly by respiratory energy conversion at low oxygen partial pressure microaerobically (Holt et al., 1994) hence able to assimilate these low molecular weight compounds. This also suggests that the photobacterium used in this study is a facultative bacterium which grows in the presence or absence of molecular oxygen. The low content of these energy rich molecules in the binary supplemented SW is an indication that ferrous and urea addition increased the bacteria activity which in turn aided the assimilation and bioconversion of majority of the organic constituents of the SW in contrast to the non-supplemented SW. The alcoholic content decreased from >4000 to <10 and 500 mgl^{-1} in both the supplemented and non-supplemented wastewater, respectively. Also, non-autoclave-sterilized SW used in this experiment could also account for the absence of the soluble fractions of organic matter in the influent wastewater.

Significance of this study on the environment

This study shows that microaerobic environmental conditions could be used to ameliorate and purify organic laden wastewater at low retention time of 3 days. Although TP level increased upon initiation of the experiment, the organism was able to assimilate it back within 72 h, resulting in 49.6% reduction. The TP increase upon initiation of the experiment could be explained in two ways, first, it could be error in analytical procedure since dissolved organic, condensed and orthophosphates, together with particulate and colloidal associated phosphates are collectively defined as total phosphate (Bensona et al., 1996). Secondly, it could be that the bacteria releases TP during the generation of energy rich chemical adenosine ATP.

The total nitrogen increased approximately from 50 to 220mgl^{-1} upon urea addition but was reduced by 67% after purification. These findings denote that the treated SW could be freely discharged into aquatic environment without fear of eutrophication or algal blooms since the residual nutrients are below limit as stipulated by World health organization (1992). Both the TOC and COD levels were also reduced by approximately 90 and 80% after 3 days treatment of the SW. In this study, inorganic nutrients were introduced as part of the SW supplement, it is worthy to note that analysis of the treated SW cum the biomass depicted that the bacteria has potential for heavy metal tolerance and assimilation (Table 4).

Conclusion

This study has demonstrated that with availability of sufficient nutrient (both micro and macronutrients), microaerobic bioremediation is a viable process for organic wastewater purification using photosynthesis bacteria at intense light radiation. It has equally shown that ferrous iron has a significant role in cell metabolism more than the tested inorganic ions (Mo^{2+} and Mg^{2+}). Also, this study supports the scenario that some organisms have potential for metal tolerance and sorption capacity as shown in Table 3 that majority of the added metals were assimilated by the bacteria. Finally, the use of microorganisms for wastewater remediation is friendly to the environment as the contaminants are converted to useful resource; however, in the present study, the crude

protein content is low (11.8%). Nevertheless, further work on this study is to carry out the experiment on a pilot scale microaerobically with real time high organic strength wastewater.

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REFERENCES

Alpha (1992). Standard Methods for the Examination of Water and Wastewater. 18th ed. American public Health Assoc. Washington, DC

Bacquevort S, Lancelot C, Schoemann V (2007). The role of iron in the bacteria degradation of organic matter derived from phaeocystis Antarctica. Biogeochemistry, 83: 119-135.

Bensona RL, Mckelvie ID, Hart BT, Truong YB, Hamilton IC (1996). Determination of total phosphorus in waters and wastewaters by on-line UWthermal induced digestion and flow injection analysis. Anal. Chem. ACT. 326: 29-39.

Bitton G (2005). Wastewater Microbiology. 3rd Edition.Wiley-Liss Pub, ISBN 0-471-30985-0. pp. 55-72, 213-222, 502-518.

Borch T, Masue Y, Kukkadapu RK, Fendorf S (2007). Phosphate imposed limitations of biological reduction and alteration on contaminant reduction of ferrihydrite. Environ. Sci. Technol. 41: 166-172.

Briggs DE, Boulton CA, Brooers PA, Stevens R (2004). Brewing science and practice. CRC press, ISBN 0-8493-2547-1. p. 364.

Chae SA, Hwang EJ, Shin HS (2006). Single cell protein production of Euglena gracilis and carbondixide fixation in an innovative photobioreactor. Bioresour. Technol. 97: 322-329.

Choorit W, Thanahoset P, Thongpradistha J, Sasaki K, Noparatnaraporn N (2002). Identification and cultivation of photosynthetic bacteria in wastewater from a concentrated latex processing factory. Biotechnol. Lett. 24: 1055-1058.

Christiansen BC, Zunic TB, Dideriksen K, Stipp SL (2009). Identification of green rust in ground water. Environ. Sci. Technol. 43: 3831-3838.

Ding C (2008). Experiments on the biodegradation of phenol wastewater by immobilized photosynthetic bacteria. Water Res. Proct. 24(6): 93-95.

Do YS, Schmit TM, Zahn JA, Boyd ES, Mora A, DiSpirito AA (2003). Role of Rhodobacter sp. strain PS9, a purple non-sulfur photosynthetic bacterium isolated from an anaerobic swine waste lagoon, in odor remediation. Appl. Environ. Microbiol. 69: 1710-1720.

Haun JKX (2005). Treating high strength antibiotic wastewater by microaerobic hydrolysis and acidification process. PubMed. (PMID: 16124480). 26(3): 106-111.

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994). Bergey's Manual of Determinative Bacteriology. 9th, Baltimore; the Williams Wilkins Co., (ISBN 0-683-00603-7). p. 787

Honda R, Fukushi K, Yamamotu K (2006). Optimization of wastewater feeding for single cell protein production in an anaerobic wastewater treatment process utilizing purple non-sulfur bacteria in mixed culture condition. J. Biotechnol. 125: 565-573.

Hong YG, Gu JD (2009). Bacteria anaerobic respiration and electron transfer relevant to biomass formation of pollutants. Int. Biodeterioration and Biodegr. 63: 973-980.

Kirchman DL, Meon B, Cottrel MT, Hutchins DA (2000). Carbon versus iron limitation of bacterial growth in the califonia upwelling regime. Limnol. Oceanog. 45: 1681-1688.

Kobayashi M, Tchan YT (1973). Treatment of industrial waste solutions and production of useful by-products using photosynthetic bacterial method. Water Res. 7: 1219-1224.

Libing C, Xingwen Z, Fenglin Y, Xiaohui L (2006). Treatment of domestic wastewater by using a micoaerobic membrane bioreactor. Desalination, 189(1-3): 181-192

Lorrungruang C, Martthong J, Sasaki K, Noparatnaraporn N (2006). Selection of photosynthetic bacterium Rhodobacter Sphaeroides 14F for polyhydroxyalkanoate production with two-stage Aerobic Dark Cultivation. J. Biosci. Bioeng. 102(2): 128-131

Madukasi EI, Dai XI, Chunhua H, Zhou JJ (2010). Potentials of phototrophic bacteria in treating pharmaceutical wastewater. Int. J. Environ. Technol. 7: 169-174.

Matsunaga T, Hatane T, Yamada A, Matsumoto M (2000). Microaerobic hydrogen production by photosynthetic bacteria in a double-phase photobioreactor. Biotechnol. Bioeng. 68(6): 647-651

Myung KK, Choi KM, Yin CR, Lee KY, Im WT, Lim JH, Lee ST (2004). Odorous swine wastewater treatment by purple non-sulfur bacteria, Rhodopseudomonas palustris, isolated from eutrophicated ponds. Biotechnol. Lett. 26: 819-822.

Okubo Y, Hiroyuki F, Akira H (2006). Characterization of phototrophic purple non-sulfur Bacteria forming colored microbial mats in a swine wastewater Ditch. Appl. Environ. Microbiol. 12(9): 6225-6233.

Padh H (1990). Cellular functions of ascorbic acid. Biochem. Cell Biol. 68: 1166-73.

Pauldyal SP, Aryal RR, Chauhan SVS, Maheshwari D (2007). Effect of heavy metals on growth of Rhizobium strain and symbiotic efficiency of two species of tropical legumes. Sci. World, 5: 27-32.

Roulston TH, Cane JH, Buchmann SL (2000). What governs protein content of pollen: Pollinator preferences, pollen pistil interactions, or phylogeny? Ecol. Monogr. 70: 617-643.

Shi XY, Yu HQ (2006). Conversion of individual and mixed volatile fatty acids to hydrogen by Rhodopseudomonas capsulatas. Int. Biodeter. Biodegr. 58: 82-88.

Wang F, Lu S, Wei Y, Min J (2009). Characteristics of aerobic granule and nitrogen and phosphorus removal in a sequencing batch reactor. J. Hazard. Mater.164: 1223-1227.

World Health Organization (1992). Draft guidelines for water quality. PCS/EHC/92.60c World Health Organization International Programme on chemical safety.

Yegani R, Yoshimura S, Kazumori M, Tomoshisa K, Shigeo K (2005). Improvement of growth stability of photosynthetic bacterium, Rhodobacter capsulatus. J. Biosci. BioEng. 100: 672-677.

Zhang QG, Zhou R, Zhang J, Yang Q (2007). Influence of light intensity on hydrogen production by R. sphaeroides. Int. J. Global Energy Issues. 28: 382-388.