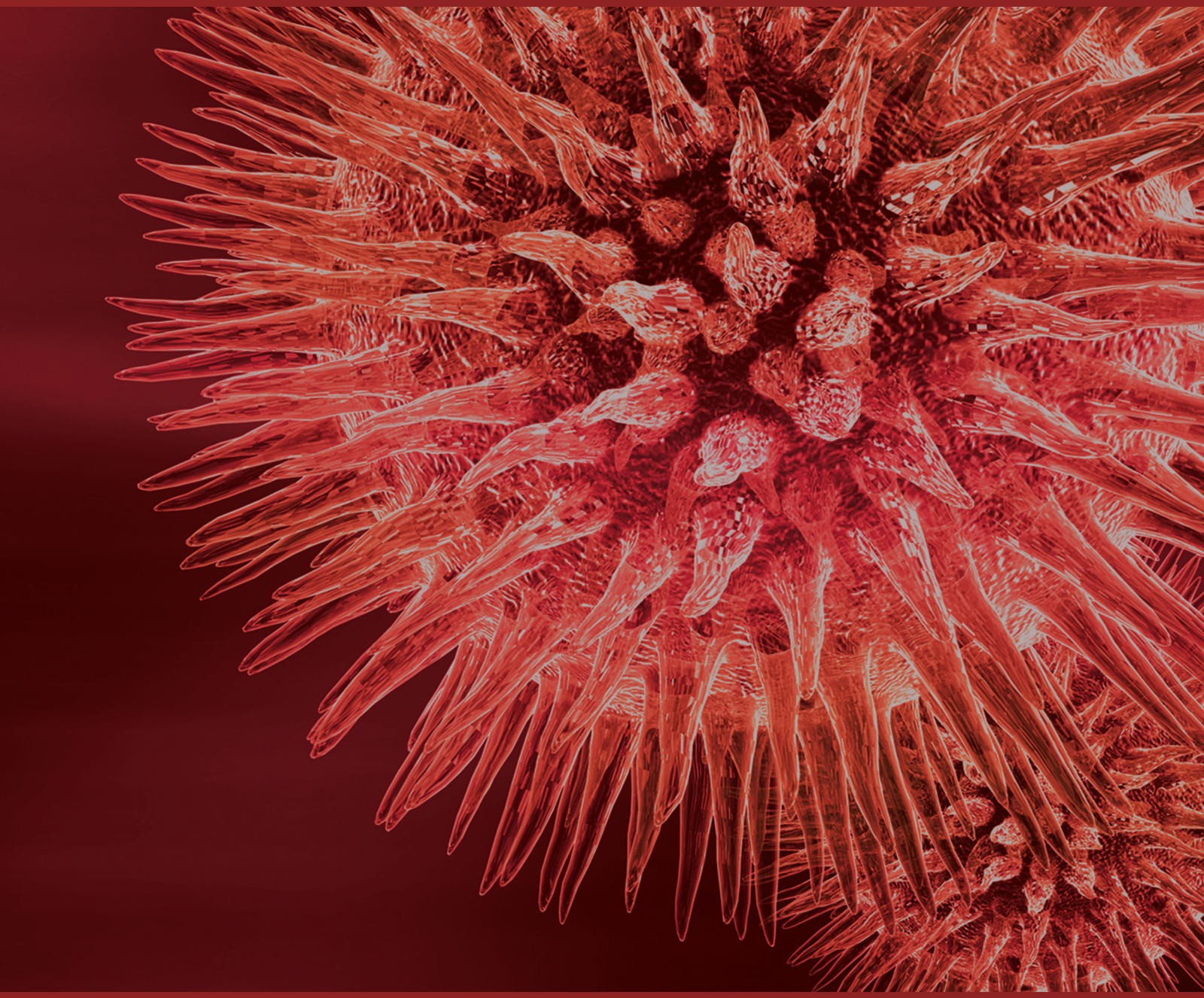
BioMed Research International

Biotechnology in Environmental Monitoring and Pollution Abatement 2015

Guest Editors: Kannan Pakshirajan, Eldon R. Rene, and Aiyagari Ramesh



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Editorial

Biotechnology in Environmental Monitoring and Pollution Abatement 2015

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1. Introduction

Rapid industrial growth has led to elevated discharges of toxic chemicals and nutrients in water bodies. The level of a particular pollutant discharged into water bodies depends on industrial activities in the vicinity. Industries such as textiles, mining, tanneries, metal plating, fertilizer and agroindustries, batteries, pesticides, ore refineries, petrochemicals, and paper manufacturing are amongst others that contribute greatly to soil, sediment, air, and water pollution problems. Some of the chemicals are not biodegradable and therefore tend to accumulate in tissues and bioaccumulate in the food chain. This results in health problems in human beings and death of aquatic organisms. In water bodies, the presence of nitrogen and phosphorus increases the production of biomass in aquatic systems, thereby impairing the water quality and threatening the natural balance of these ecosystems. Although stringent nitrogen and phosphorus discharge standards from wastewater have been set in many countries, industries often face problems in meeting these requirements. From the regulatory perspective of a particular country, it is necessary to develop new or optimize the existing wastewater treatment technologies for compliance with the latest discharge standards.

The demand for the use of sustainable and ecofriendly environmental processes is rapidly growing subjected to economic, public, and legislation pressure. Biotechnology provides a plethora of opportunities for effectively addressing issues pertaining to the monitoring, assessment, modeling, and treatment of contaminated soil, sediment, air, and water streams. The different biotechnologies available nowadays represent both well-established and novel (bio)technologies, although several aspects of their performance remain to be tested, for instance, the use of novel biocatalysts and reactor designs, a fundamental understanding of microbial community dynamics and mechanisms occurring within a (bio)reactor, the assessment of the performance of (bio)reactors during long-term operation, and its modeling [1–6]. If these mechanisms are understood and the knowledge gap is bridged, novel biotechniques will potentially change the way users rebuild technologies for the sustainable use of different biological processes for soil, sediment, air, and wastewater treatment.

2. Industrial Wastewater Treatment

Agricultural runoff, livestock operations, aquaculture, food processing facilities, pulp and paper mills, sewage treatment plants, and fossil fuel combustion are some typical examples of industries that cause nutrient pollution. In agricultural areas, N, P, and K compounds are easily transported by farmland drainage and surface water to valuable water resources resulting in the deterioration of water quality. C/N ratio is an important parameter to design biological wastewater

Table 1

Title of the accepted manuscript	Authors
"Characteristics of Biological Nitrogen Removal in a Multiple Anoxic and Aerobic Biological Nutrient Removal Process"	H. Wang et al.
"Evaluation of Natural Materials as Exogenous Carbon Sources for Biological Treatment of Low Carbon-to-Nitrogen Wastewater"	J. Ramírez-Godínez et al.
"Decolorization of Distillery Spent Wash Using Biopolymer Synthesized by *Pseudomonas aeruginosa* Isolated from Tannery Effluent"	C. David et al.
"Enhancing Ecoefficiency in Shrimp Farming through Interconnected Ponds"	R. H. Barraza-Guardado et al.

Table 2

Title of the accepted manuscript	Authors
"Integrated Evaluation of Urban Water Bodies for Pollution Abatement Based on Fuzzy Multicriteria Decision Approach"	S. Hashim et al.
"Vulnerability Assessment and Application of Bacterial Technology on Urban Rivers for Pollution Eradication"	S. Hashim et al.
"The Regulation by Phenolic Compounds of Soil Organic Matter Dynamics under a Changing Environment"	K. Min et al.

treatment systems, particularly for treating high N containing wastewater. Sometimes, for unbalanced C/N ratios, exogenous carbon sources can be added to the wastewater. Low cost biological matrices such as woodchips, peanut shells, and barley grains can be added as potential carbon donors. With regard to the use of bioreactors for nitrogen removal, sequencing batch reactors (SBRs) can be used to achieve nitrification under aerobic conditions and denitrification under anoxic conditions. In order to accomplish enhanced nitrogen removal in SBRs, activities of both nitrification and denitrification should be studied during long-term system operation. Table 1 presents the papers accepted under this category and their authors.

3. Control and Assessment of Environmental Pollution

It is a well-known fact that sewage water from various sources is discharged into urban rivers or streams. Conventionally, the water quality index approach is one of the best tools to determine the water quality of the water bodies. Recent studies have offered ingenious and innovative solution for the rehabilitation of urban water bodies and to improve their quality. Bacterial technology (BT) can rehabilitate urbanized water bodies such as lakes, rivers, and streams. BT offers the following advantages; (i) sustainable and reliable for public health, (ii) low maintenance, (iii) minimal operational costs, and (iv) reproducible on any scale of the operation. Titles of papers accepted under this category along with the authors are represented in Table 2.

Concerning the presence of phenolic compounds in soils, they are the most abundant plant metabolites and are believed to decompose slowly in soils compared to other soil organic matters. One of the papers selected for this special issue reviews the turnover rate of phenolics and its quantification in soils, together with their regulatory effects on decomposition. The authors reviewed the following aspects: (i) various structures and forms of phenolics in soils; (ii) extraction and analysis of phenolics in soil samples; (iii) phenolics biodegradation; (iv) effect of phenolics on soil organic matter decomposition; (v) effect of environmental changes, such as elevated CO_2, global warming, N deposition, and drought, on phenolics decomposition; and (vi) suggestions for future phenolics studies.

The guest editors firmly believe that the collection of papers presented in this special issue will stimulate interest amongst the global research community and would help peers in their research pursuits. Besides, there is an urgent need to translate most of the laboratory-based research into field-based research in order to witness sustainable solutions to persisting environmental problems. Future research should address crucial issues pertaining to (i) nanobiomatrices for environmental remediation, (ii) development of biosensors for environmental monitoring, (iii) development of new biocatalysts (bacteria, fungi, and algae) for environmental applications, (iv) clean practices and development of technologies for pollution prevention, and (v) studies on life cycle assessment (LCA), risk assessment, health, and safety impact assessment.

Acknowledgments

The guest editors wish that this special issue will make a good reference material and be of great use for practicing environmental engineers and researchers. The guest editors thank all the authors for their generous support and dedication and for submitting high-quality papers. The final outcome of this special issue would not have been possible without the support of expert reviewers for contributing their knowledge and providing critical insight during the review process.

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Kannan Pakshirajan
Eldon R. Rene
Aiyagari Ramesh

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Research Article

Enhancing Ecoefficiency in Shrimp Farming through Interconnected Ponds

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The future development of shrimp farming needs to improve its ecoefficiency. The purpose of this study was to evaluate water quality, flows, and nitrogen balance and production parameters on a farm with interconnected pond design to improve the efficiency of the semi-intensive culture of *Litopenaeus vannamei* ponds. The study was conducted in 21 commercial culture ponds during 180 days at densities of 30–35 ind m^{-2} and daily water exchange <2%. Our study provides evidence that by interconnecting ponds nutrient recycling is favored by promoting the growth of primary producers of the pond as chlorophyll *a*. Based on the mass balance and flow of nutrients this culture system reduces the flow of solid, particulate organic matter, and nitrogen compounds to the environment and significantly increases the efficiency of water (5 to 6.5 m^3 kg^{-1} $cycle^{-1}$), when compared with traditional culture systems. With this culture system it is possible to recover up to 34% of the total nitrogen entering the system, with production in excess of 4,000 kg ha^{-1} shrimp. We believe that the production system with interconnected ponds is a technically feasible model to improve ecoefficiency production of shrimp farming.

1. Introduction

The future development of shrimp farming requires innovative and responsible practices to improve their operating efficiency and help prevent environmental degradation of coastal ecosystems [1]. Some proposals include the use of mangroves as biofilters of crop effluents [2], performing polycultures with seaweed and shellfish [3, 4], the use of microbial mats in ponds [5], farming systems with low water exchange [6], and strategies for cleaner power [7]. Exchange or recycling of the water in the ponds serves to keep the water variables in conditions suitable for the growth and development of shrimp. However, rates of over 16% water exchange increase operating costs, such as the amount of fuel used, as well as increasing the quantity of pollutant inputs [8]. Semi-intensive shrimp farming of northwestern Mexico can have water exchange rates greater than 25% water [9], but mass mortality events in 2010, 2011, 2012, and 2013 due to the presence of diseases recommend reducing water turnover rates [10]. In aquaculture systems with low water turnover rates autotrophic, chemoautotrophic, and phototrophic processes have been studied, and a rapid increase in organic matter has been observed, which can serve as a substrate for the development of heterotrophic bacteria; on the other hand

nitrogen compounds are remineralized by nitrifying bacteria and are consumed by microalgae. These processes allow for potentially polluting compounds to enter the food chain [11–14]. High turnover rate allows for some water quality variables to be well regulated in terms of water quality; it nevertheless represents a massive waste of potentially useful nutrients and organic matter. Martínez-Córdova et al. [15] demonstrated experimentally that it is possible to reuse the effluent of semi-intensive ponds to grow bivalves, benthic diatoms, and whiteleg shrimp (*Litopenaeus vannamei*) in a multitrophic system. Nevertheless, this practice requires validation for use on a commercial scale because the effects on water quality and productive performance of shrimp, as well as N recycling and discharge, are unknown. It is widely documented that only between 18 and 27% of N entering the ponds is converted into shrimp biomass; the rest is discharged into the environment [16–19]. Most of N entering the ponds exits through effluents during water changes. Water exchange in addition to influencing discharge potentially releases harmful components for the environment, representing huge volumes of water masses that move annually between coastal water bodies and fish farms. In the northwest of Mexico, shrimp farming systems use ~57 $m^3\,kg^{-1}$ water shrimp [13, 19]. The effects of having excessive discharges of effluent from shrimp farms include organic enrichment of the sediment and water, hypernutrification and discharge of high concentrations of heterotrophic bacteria, nitrifying, and types of vibrio [20]; such alterations influence the distribution and abundance of benthic species [21]. In the northwest of Mexico, the recovery of N is 25 to 35%, and discharge is from 27 to 35% with water exchange rates that can exceed 16% daily [9, 19]. Our hypothesis is that farming systems can reduce turnover rates and leverage the recycling of nutrients in order to promote ecoefficiency in shrimp farming. The study was conducted on a farm in semi-intensive shrimp farming designed with interconnected ponds (unique to Mexico) for reuse; water exchange rates <2% water. The goal was to evaluate the effect of this interconnected pond design with low water exchange rates on water quality, production parameters, material flows, and nitrogen contribution to the environment.

2. Materials and Methods

2.1. Area of Study. Shrimp aquaculture farm Acuícola Polo, S.A. de C.V., is located in northwest Mexico (Figure 1). The farm consists of three modules; Module 1 (M1) has 34 rectangular earthen ponds 1 ha. each (average depth 1.2 m, volume of 12,000 m^3), and Module 2 (M2) has 30 ponds of the same depth and volume as that of the M1, and Module 3 (M3) has 10 ponds of three ha. each (average depth of 1.5 m and volume of 46,500 m^3).

The water was pumped directly from an inlet open to the sea and channeled to two reservoirs (reservoir 1 and reservoir 2). From these channels reservoirs, water flowed from the first to the last tank by plastic tubes (Figure 1). The tanks of each module were maintained interconnected by plastic tubes of 1 m diameter placed along the edge perimeters of the ponds (Figure 1). In each module the water flowed through the first pond and then flowed into the other and so forth until reaching the last pond. This design allowed foe water reuse from the first pond to the last throughout the crop cycle (Figure 1).

Water exchange rates were performed daily with a percentage of 1.6 ± 0.24% for modules 1 and 2 and 1.5 ± 0.22% for the M3. The estimates of water exchange rates were determined following the criteria of Wheaton [22].

In M1, M2, and M3 postlarvae of *L. vannamei* (PL_{14}, average weight 1.1 mg) were seeded at densities of 30, 30, and 35 PL/m^{-2}. The days of culture in both modules were 187 and 157 days. During this period the shrimp were fed daily three times a day (08:00, 14:00, and 20:00), with commercial feed (35% crude protein, 88% dry matter, and 8% lipids). The daily ration was estimated according to [9, 23]. During cultivation no fertilizer was added to the ponds.

2.2. Water Quality. The water quality parameters were monitored in the pumping station (a), reservoirs (b), and the water outlet for each of the 20 ponds studied (c) in the three modules (Figure 1).

At each sampling site temperature was recorded daily, as well as dissolved oxygen (DO) and salinity with YSI multisensor (Model YSI 85, YSI Incorporated, Yellow Springs, Ohio 45387 USA) and pH with a potentiometer Model Hanna 220A. Each week water transparency was recorded with a Secchi disk. Every two weeks, water samples were collected in 1 L plastic bottles to determine suspended solids (inorganic and organic) nutrients, chlorophyll *a*. Water samples were kept on ice during transport to the laboratory.

2.3. Total Suspended Solids, Particulate Organic Matter, and Chlorophyll a. The water samples were filtered through a vacuum pump through glass fiber filters Whatman GF/C of 47 mm diameter and 1.4 μ pore opening. To determine suspended solids and organic matter the Strickland and Parsons technique [24] was carried out. Chlorophyll *a* was determined with the procedure of Parsons et al. [25] using 90% acetone for removal of pigments.

2.4. Dissolved Inorganic Nutrients. Previously filtered water was used to determine the concentration of dissolved inorganic nutrients (NO_2-N, NO_3-N, and NH_4-N) by a spectrophotometer Hach DR/5000 using methods of diazotization with ferrous sulfate acid medium (Method 8507) for NO_2^--N, cadmium reduction to NO_2^--N, and diazotization (Method 8171) for NO_3^--N and salicylate (Method 8155) for NH_4^+-N following the procedure described in the manual [26].

2.5. Total Nitrogen by Kjeldahl Method. Water samples collected were processed in triplicate following the micro Kjeldahl method that included digestion with sulfuric acid and hydrogen peroxide, according to the method 8075 of procedures spectrophotometer manual [26].

2.6. Chemical Flows and Partial Mass Balance Calculation. The estimate of the daily nutrient water quality was obtained

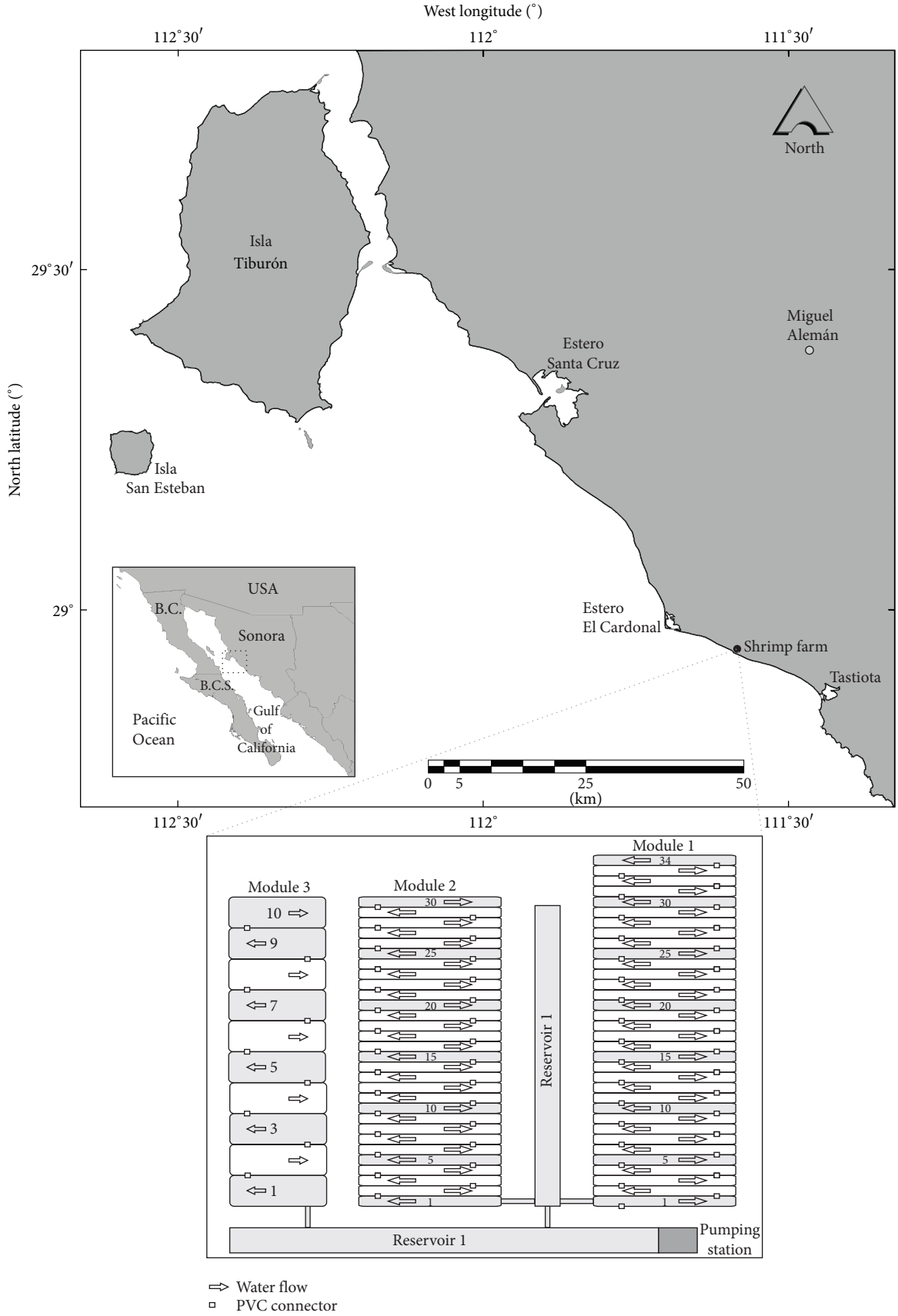

Figure 1: Study shrimp farm location and design of three modules with interconnected ponds. The arrows show the flow of water between the ponds. Studied ponds are designated with numbers.

with weekly data interpolation [19]. The concentrations were multiplied by the daily water exchange to determine the total weight of each parameter in the exchanged water. Similarly, the mass flow of each parameter which entered and exchanged through the ponds was calculated based on water entering from the harbor. The net water balance (kg ha^{-1}) was estimated from the difference between the inputs and outputs [19].

Farm records were used to quantify the amount of food added to each pond. The concentration of nitrogen in the feed used and shrimp harvested was calculated according to [16, 19].

To estimate the nitrogen content in the associated macrofauna we used the average value reported in studies of [16, 27–29].

The volume of refilled water is based on the records of the farm. Evaporation and precipitation were estimated based on the records of the weather station of the National Water Commission for the Costa de Hermosillo Sonora Mexico [30].

Water flows were calculated based on volumes of water exchange rate, evaporation, and precipitation. The inputs of nutrients via atmospheric precipitation and nitrification and fixation of nutrients by microalgae were not considered for this study. Estimates of flows admission and release of N were expressed in kg ha^{-1} $cycle^{-1}$.

2.7. Statistical Analysis. Tests for homoscedasticity and normality were applied to determine the use of parametric or nonparametric methods [30, 31]. ANOVA Kruskal-Wallis was used to determine differences between the levels of the variables of water and they were used to evaluate production parameters of the three modules studied. In cases in which there were significant differences, multiple comparisons tests were run. In all cases the level of significance was 0.05. The data were processed using the Number Cruncher Statistical System software [32].

3. Results and Discussion

In this culture model with low turnover and reuse of water, shrimp growth was not limited by the quality of water variable, keeping water quality within safe levels [33, 34]. The averages of the variables of water during the growing season are presented in Table 1. Concentrations of DO in a few of the weeks in the mornings were below recommended levels (<2 mg L^{-1}), this is because no mechanical aeration was used in addition to the combined effect of high natural productivity, temperature, and salinity prevailing in the water during this period. The average dissolved oxygen varied from 2.8 mg L^{-1} (morning) to 6.3 mg L^{-1} (afternoon). Some studies show that values < 2 mg L^{-1} of DO can be critical for the growth of shrimp [35, 36], but in our study no mortalities were observed. Comparatively, M1 and M2 had similar water quality conditions, while M3 had higher water temperature since cultivation began a month later, the DO was lower and the NH_4-N was the highest and this is mainly attributed to the higher planting density (35 PL m^{-2}).

Lower salinity values (37.9 psu) were recorded at beginning of cultivation, while at the end values reached 45 psu. However, the low rate of water exchange salinity had a small increase (9 psu) and remained at levels comparable to other studies in shrimp farms in Northwestern Mexico: 42 to 48 psu [19], 45 ± 5 psu [13], and 41 to 42 psu [37].

Production results are presented in Table 2. The survival rate varied between 70.9 and 78.0% with an average weight that was between 17 and 20 g, with no significant difference between the modules. Shrimp production for M1, M2, and M3 was 4,285, 4,250, and 4,683 kg ha^{-1}, respectively (Table 2).

The concentrations of nitrogen compounds (NH_4-N and NO_2-N, NO_3-N) remained at comparable levels to those seen in a traditional semi-intensive culture of *L. vannamei* in Northwestern Mexico, where Casillas-Hernández et al. [34] reported 0.1 to 0.1 mg L^{-1} of NH_4-N and 0.05 mg L^{-1} of NO_2-N 0.5 mg L^{-1} of NO_3-N while Miranda et al. [13] reported 0.1 mg L^{-1} of NH_4-N 0.04 mg L^{-1} NO_2-N and 0.1 mg L^{-1} of NO_3-N. The concentrations of NO_3-N observed in this study >1 mg L^{-1} are consistent with previous studies on farms in the regions ~2 mg L^{-1} [38] and ~3 mg L^{-1} [37]. These levels of NO_3-N indicate an efficient nitrification within farming systems [39].

TNK concentration observed was similar to those reported by Miranda et al. [13]. ~2 mg L^{-1} suggested that the system of interconnected ponds has low water exchange rate but provided efficient remineralization of N. Dissolved inorganic nitrogen (DIN = NO_2-N + NO_3-N + NH_4-N) maintained average concentrations >1 mg L^{-1} similar to that observed by Wang et al. [40] ~2 mg L^{-1} for intensive cultivation of *L. vannamei* (62–227 ind m^{-2}) in ponds treated with probiotics. This indicates that the interconnected ponds can act as remineralization lagoons where the accumulation of N can promote the development of natural productivity.

The biomass of phytoplankton in the ponds was higher in the middle sections and at the end of the modules studied, indicating a higher level of eutrophication as water was being reused. Evidence of this was provided by the concentration of Chlorophyll *a* in our study which was higher than those reported for crops of traditional semi-intensive systems for *L. vannamei* in Mexico, which were 10 ± 8 mg m^{-3} [19] and 6 ± 3 mg m^{-3} [13] and 15 ± 1 to 17 ± 2 mg m^{-3} [34] and 8 ± 3 to 16 ± 2 mg m^{-3} [37].

Total suspended solids, from both inorganic and particulate organic matter showed similar concentrations between M1, M2, and M3 and are within a range comparable with that reported for semi-intensive culture of *L. vannamei* in Mexico TSS: 96 ± 5, SSI: 69 ± 36 and POM: 27 ± 7 mg L^{-1} [13], TSS: 124 ± 11 to 153 ± 12, and POM: 30 ± 3 to 38 ± 3 mg L^{-1} [34]. Our results showed that the culture system of interconnected ponds maintained a proper process of remineralization of organic matter which was provided by shrimp feces and leftover food, so nutrients helped keep significant concentrations of phytoplankton biomass, promoting the presence of natural food in the culture system.

One way to assess the efficiency of water is estimating the volume of water used to produce one kg of shrimp per crop

Table 1: Mean value (±SD) of water quality variables in three modules during a 157–187-day trial.

Water quality variable	M1	M2	M3	P
Temperature (6 h°C)	28.3 ± 2.59^{a}	28.1 ± 2.52^{a}	29.3 ± 2.0^{b}	<0.001*
Temperature (14 h°C)	30.7 ± 2.14^{a}	30.9 ± 2.18^{a}	31.3 ± 2.08^{b}	<0.001*
DO. (6 h mg L^{-1})	3.57 ± 1.53^{b}	3.58 ± 1.43^{b}	2.81 ± 1.73^{a}	<0.001*
DO. (14 h mg L^{-1})	5.45 ± 1.52^{b}	6.37 ± 1.0^{c}	4.72 ± 1.65^{a}	<0.001*
Salinity (‰)	38.7 ± 3.24^{a}	38.5 ± 3.0^{a}	40.4 ± 5.53^{a}	0.28
pH (14 h)	8.22 ± 0.22^{a}	8.2 ± 0.21^{a}	8.19 ± 0.28^{a}	0.40
Transparency (14 h cm)	38.5 ± 13.5^{a}	39.7 ± 9.8^{a}	46.6 ± 19.1^{b}	<0.001*
TSS (mg L^{-1})	128.4 ± 49.7^{a}	126.2 ± 54.7^{a}	173.0 ± 52.0^{a}	0.31
ISS (mg L^{-1})	108.2 ± 45.1^{a}	106.5 ± 48.8^{a}	116.6 ± 45.9^{a}	0.24
POM (mg L^{-1})	20.4 ± 8.4^{a}	19.9 ± 8.0^{a}	20.4 ± 8.8^{a}	0.89
Chlorophyll *a* (mg m^{-3})	32.4 ± 15.8^{ab}	38.4 ± 20.0^{b}	29.1 ± 21.1^{a}	0.02*
NO_2-N (mg L^{-1})	0.0043 ± 0.0021^{a}	0.0043 ± 0.0024^{a}	0.0062 ± 0.0058^{a}	0.06
NO_3-N (mg L^{-1})	1.4 ± 0.57^{a}	1.3 ± 0.49^{a}	1.37 ± 0.37^{a}	0.40
NH_4-N (mg L^{-1})	0.08 ± 0.05^{a}	0.07 ± 0.05^{a}	0.11 ± 0.05^{b}	<0.001*
TNK (mg L^{-1})	2.5 ± 1.3^{ab}	2.14 ± 1.1^{a}	2.8 ± 1.11^{bc}	<0.001*

Different letters among modules for each variable indicate significant differences, * indicate probablity: ANOVA Kruskal-Wallis, and $P < 0.05$.

Table 2: Water exchange, survival, final body weight, total production, and feed conversion ratio per module during trial *Litopenaeus vannamei*.

Variables	M1	M2	M3
Water exchange day (%)	1.6 ± 0.24	1.6 ± 0.24	1.5 ± 0.22
Survival (%)	74.8 ± 7.6^{a}	70.9 ± 4.7^{a}	78.0 ± 6.7^{a}
Day of trial	187	187	157
Stocking density (PL/m^2)	30	30	35
Water flow (m^3 ha^{-1} $cycle^{-1}$)	27,700	27,700	24,100
Final body weight (g)	19.14 ± 0.69^{b}	20.0 ± 0.82^{b}	17.17 ± 0.88^{a}
Production (Kg ha^{-1})	4285 ± 292^{a}	4250 ± 202^{a}	4683 ± 384^{b}
Feed added (Kg ha^{-1} $cycle^{-1}$)	7801 ± 282	8074 ± 242	8115 ± 495
Feed conversion ratio	1.82 ± 0.11^{a}	1.9 ± 0.04^{a}	1.74 ± 0.18^{a}

Different letters among modules for each variable indicate significant differences (ANOVA Kruskal-Wallis, one via $P < 0.05$).

cycle. In our study the efficiency was 5 to 6.5 m^3 kg^{-1} $cycle^{-1}$ at 160 to 190 days of culture, which is significantly lower compared to other semi-intensive crops. Reference [41] estimated a worldwide range of 39 and 199 m^3 kg^{-1} $cycle^{-1}$ for semi-intensive and intensive shrimp culture systems, respectively. Until recently values 100–200 m^3 kg^{-1} $cycle^{-1}$ were considered to be efficient for semi-intensive systems [42]. In Northwest Mexico, semi-intensive systems have shown a broad range [38] and obtained an average of 45 m^3 kg^{-1} $cycle^{-1}$ with 7% daily water exchange; Casillas-Hernández et al. [9] obtained 62–71 m^3 kg^{-1} $cycle^{-1}$ with daily turnover of 11%; Miranda et al. [13] reported values of 101–105 m^3 kg^{-1} $cycle^{-1}$ with a turnover of 13% day^{-1}. Studies in shrimp cultures with low water exchange in Mexico have reported rates of 9 to 17 m^3 kg^{-1} $cycle^{-1}$ with 3–5% daily turnover [19], 17 to 38 m^3 kg^{-1} $cycle^{-1}$ with 5% daily turnover in 140-day cycles [15], and 17 to 21 m^3 kg^{-1} $cycle^{-1}$ with 5% daily turnover in of 120-day cycles [37].

The feed conversion factor (FCF) obtained in the present study (Table 2) was lower than that reported (2.2) by Miranda et al. [13] and remained within the range (1.2 to 1.8) obtained by Páez-Osuna et al. [19] in farms in the Northwest of Mexico. The global average of FCA for semi-intensive shrimp farms is 1.8 [6, 15]. This indicates that the administration and feed efficiency in our study was similar to that obtained in traditional farms, but with more efficient use of water, thus improving overall efficiency since it promotes recycling of nutrients in ponds and increases primary productivity. This has been observed previously by [43] that it is feasible to reduce the food conversion factor.

The evaluated model of interconnected ponds with low water exchange was more efficient because it exported less volumes of TSS (660 to 1,566 kg ha^{-1}), ISS (441 to 1,280 kg ha^{-1}), POM (221 to 407 kg ha^{-1}), TON: total organic nitrogen (12–36 kg ha^{-1}), and TIN: total inorganic nitrogen (8–15 kg ha^{-1}) compared to other reports from semi-intensive farms in Mexico that operate with traditional ponds; TSS: 12,696 to 17,539, POM: 3,054 to 5,349, and TIN: 18.6 to 20.8 kg ha^{-1} [34]; TSS: 8,479, ISS: 7,562, POM 917, TON: 103, and TIN: 19 kg ha^{-1} [13]. In our study net contributions of materials are similar to that observed in cultures operated with lower stocking densities (14 to 20 ind m^{-2}) and turnover rates of 3 to 5%, TSS: 1591 and POM: 199 kg ha^{-1} [19].

Net discharges of the materials per kg of shrimp produced (TSS: 0.16 to 0.37, ISS: 0.1 to 0.3, MOP: 0.1, TON: 0003 to 0008, TIN: 0.002 to 0003, and chlorophyll *a*: 0.0001 to 0.0003 kg^{-1} shrimp) also found that the tested model has better efficiency than traditional semi-intensive crops TSS: 4.2, ISS: 3.8, POM: 0.5, TON: 0.1, TIN: 0.01, and chlorophyll *a*: 0.0005 kg^{-1} shrimp [13] and TSS: 4.3 to 5.3, POM: 0.9 to 1.8, TIN: 0.006, and chlorophyll *a*: 0.001 kg^{-1} shrimp [34].

Table 3 presented the N mass balance calculations for each of the modules. In each case the most important source of N to the system was from the artificial food in M1 (82%),

Table 3: Partial nutrient budget N, for different modules during a 157–187-day trial.

Variables	(kg ha^{-1} cycle^{-1})	(%)
Module 1		
Feed shrimp	384.43	81.78
Postlarval shrimp	0.02	<0.01
N-inorganic	28.86	6.14
N-organic (TKN)	56.79	12.08
Total input	470.10	100.0
Macrofauna	1.1926	0.2537
Biomass shrimp	146.09	31.07
N-inorganic	39.96	8.50
N-organic (TKN)	63.76	13.56
Sedimentation and volatilization	219.09	46.61
Total output	470.10	100.0
Module 2		
Feed shrimp	397.88	83.43
Postlarval shrimp	0.01987	0.004
N-inorganic	30.55	6.41
N-organic (TKN)	48.46	10.16
Total input	476.92	100.0
Macrofauna	0.9228	0.1935
Biomass shrimp	144.87	30.37
N-inorganic	35.53	7.45
N-organic (TKN)	65.35	13.70
Sedimentation and volatilization	230.24	48.28
Total output	476.92	100.0
Module 3		
Feed shrimp	399.92	84.33
Postlarval shrimp	0.01987	0.004
N-inorganic	27.13	5.72
N-organic (TKN)	47.14	9.94
Total input	474.22	100.0
Macrofauna	1.2483	0.1935
Biomass shrimp	159.62	33.66
N-inorganic	37.53	7.91
N-organic (TKN)	72.73	15.34
Sedimentation and volatilization	203.09	42.83
Total output	474.22	100.0

M2 (83%), and M3 (84%). Organic N input from water for M1 accounted for 12% and for M2 and for M3 10%. The inorganic N for M1, M2, and M3 was 6%. The N content in postlarvae was almost negligible (<0.1% in all the three modules).

According to the mass balance results the greatest loss of N was via sedimentation and volatilization of ammonium; values in the modules M1, M2, and M3 were 47%, 48%, and 43%, respectively. The amount of N removed during harvest shrimp in M1, M2, and M3 was 31%, 30%, and 34%, respectively. The discharge of effluent via organic N for M1, M2, and M3 represented 14% and 15%. The inorganic N accounted for and was 8% in all modules. The amount of N removed by the associated macrofauna was <0.3% in all ponds. The various inflows and outflows of N are presented in Table 3. The mass balance results indicated that the supplied food was the main N input source to the system (82–84%) (Figure 2). This coincides with previous reports for intensive and semi-intensive systems where food can contribute between 71 and 97% of total N [19, 34, 36, 44–46]. With regard to sources of N discharge, the other studies mentioned above are consistent with those observed in our study, where the main forms of N are found in the sedimentation and are volatilized in the form of ammonia. The N retrieved vis-à-vis biomass harvested shrimp was 30 to 34%, which suggests better usage of N in the food provided. Other traditional semi-intensive shrimp farms in the Northwest of Mexico reported values of 20–24% [47]. In other countries values of N vary from 18 to 27% [12, 16–18]. Based on our results, the modular design of interconnected ponds with low turnover and reuse of water significantly improves the recovery of N as shrimp tissue (Figure 2). The N sedimented and volatilized were not quantified separately; however it is possible that most of the N that had been deposited in the pond sediment is in the form of organic nitrogen sequestered in organic matter, considering that in this design the flow of water is very low favoring sedimentation in the ponds. It is assumed that the organic N in sediment was the most abundant form since sedimentation of organic matter was caused by the sum of accumulated leachated commercial feed and shrimp feces as suggested [11, 36]. Ammonia volatilization is not considered a significant loss in ponds when ammonia levels are <1 mg L^{-1} and pH 7.5–8.5 [48–50] as observed in our study. In addition, [12] mentions that the wind or mechanical ventilation are other factors that influence the presence and volatilization of ammonia (NH_3-N). Our results showed that the dominant species and chemistry in the aquaculture system was NH_4-N. This indicates a low loss by volatilization because ponds were not aerated. The organic form of N in the water was the most abundant, which coincides with Jackson et al. [12] where they reported a close relationship between chlorophyll *a* and particulate organic N, assuming that most of POM is due to the presence of phytoplankton.

Modular design in low turnover and high water retention time allowed complete nitrification. This is reflected by elevated levels of NO_3-N. The levels of organic N were similar to what was reported (~2 mg L^{-1}) by [13] although in their study turnover rate was 12% day^{-1}. In our aquaculture system with low water exchange, the low levels of NH_4-N indicate efficient nitrification, but these conditions are difficult to achieve in shrimp farms with high water exchange rate [13].

In Table 4 the nutrient flows and material discharge via water is presented. The greatest discharges corresponded to TSS (660 to 1,566 kg ha^{-1}); the ISS varied from 441 to 1,280 kg ha^{-1} and MOP 221 to 407 kg ha^{-1}. Chlorophyll *a* values varied from 0.50 to 1.27 kg ha^{-1}. The contribution from nitrogen compounds was dominated by TNK with interval of 12 to 36 kg ha^{-1}, followed by NO_3-N 6.75 to 14.8 kg ha^{-1}, NH_4-N 0.35 to 0.92 kg ha^{-1}, and NO_2-N 0.03 to 0.08 kg ha^{-1}. In all three modules the organic N (TNK) exceeded inorganic N levels. In our study, the estimated net N contribution to the environment was 24 kg ton^{-1} at shrimp planting densities of

Table 4: Fluxes estimated (kg ha^{-1}) (mean ± standard error) of incorporated, discharged, and net loading material (outlet − inlet) via water for shrimp culture in three modules.

Variables	M1			M2			M3		
	Inlet (kg ha^{-1})	Outlet (kg ha^{-1})	Net load (kg ha^{-1})	Inlet (kg ha^{-1})	Outlet (kg ha^{-1})	Net load (kg ha^{-1})	Inlet (kg ha^{-1})	Outlet (kg ha^{-1})	Net load (kg ha^{-1})
NH_4^+-N	1.91	2.26	0.35	1.48	2.40	0.92	2.26	2.75	0.49
NO_2^--N	0.08	0.12	0.04	0.08	0.11	0.03	0.11	0.19	0.08
NO_3^--N	26.9	40.7	13.7	29.0	35.7	6.75	29.2	44.1	14.8
TKN	56.7	68.7	11.9	48.4	70.4	21.9	54.8	91.1	36.3
TSS	2750.3	4316.5	1566.2	2944.7	3605.3	660.5	3045.3	4556.6	1511.3
ISS	2407.3	3688.1	1280.7	2556.8	2998.1	441.3	2669.1	3784.3	1115.1
POM	342.9	660.0	317.0	387.9	608.9	221.0	376.1	783.9	407.7
CL *a*	0.50	1.00	0.50	0.55	1.19	0.64	0.35	1.62	1.27

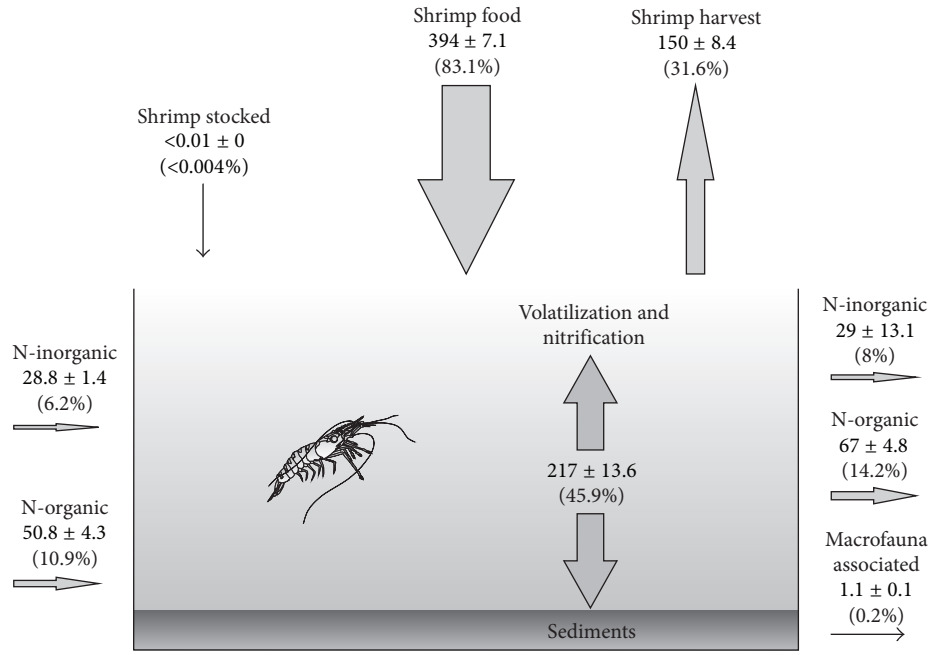

Figure 2: Mass balance for nitrogen in shrimp farm with interconnected ponds. Units in kg ha^{-1} cycle^{-1} (±SD) and parenthesis; values represent mean percentage of variables.

30–35 ind m^{-2}. Whether N loss in semi-intensive culture with *L. vannamei* is variable depending on planting density (ds), the rate of water exchange (tr), and days in culture (dc), for example, 18 kg N, ds: 11 ind m^{-2}, tr: 4.7%, and dc: 95–162 days [51]; 29 kg N, ds: 17 ind m^{-2}, tr: 3–5%, and dc: 95–165 days [19]; and 72 kg N, ds: 15 ind m^{-2}, tr: 11%, and dc: 203 days [9]. The levels obtained in this study were only surpassed by the study in [51] but with a considerably lower density. Therefore the system of interconnected ponds with low turnover had a lower environmental N loss. Environmental losses of N in intensive shrimp farming of *L. vannamei* vary between of 38–44 kg N ton^{-1} [44], 53 kg N ton^{-1} [52], and 72 kg N ton^{-1} [12]. This provides evidence that cropping systems with reduced or no turnover rate can help reduce significantly N discharge to the environment and its productions are comparable to those systems that handle high turnover rates.

Our study provides evidence that by interconnecting ponds nutrient recycling is favored. Construction engineering with interconnected ponds promotes the growth of primary producers such as pond microalgae [20], which produce sugars, proteins, and other components required by shrimp for various biochemical processes such as respiration, digestion, and biosynthesis, as well as the energy required for movement and nutrition [53]. This has a practical benefit because it can improve the conversion factor of artificial food for shrimp biomass.

The best recycling of nutrients and the promotion of microalgae also favor the development of heterotrophic

microorganisms that feed primarily on organic matter in the culture ponds [54]. This web-established food in the ponds made nutrient recycling more efficient [55], with additional practical benefits. Hence, with this water quality cropping system, nutrition and health status of the shrimp are improved [54, 56].

As previously noted in this study, the system of ponds interconnected with low turnover rates significantly increases the reuse and efficiency in water use providing economic benefits (cost savings of retail electricity and water booster factor reduction FCR) and environmental benefits (healthier aquaculture systems and crop effluent with lower contribution of important nutrients and organic matter).

We believe that the interconnection of ponds is a production model technically feasible and is compatible with other biotech innovations, for example, the implementation of bioreactors in cropping systems to facilitate the growth of beneficial bacteria consortia. In short, the study results provide elements to reduce production costs of systems of semi-intensive shrimp farming in Mexico, while also reducing environmental impacts.

In a recent review [57], it is mentioned that aquaculture must have the best practices of cultivation and the ecosystem approach to better integrate aquaculture in inland basins and coastal areas with more efficient use of land and water.

4. Conclusions

Our study provides evidence that by interconnecting ponds with low water exchange then nutrient recycling is favored and promotes growth of the food web with organisms working in nutrition and semi-intensive production of *Litopenaeus vannamei*.

According to the mass balance and flow of nutrients this culture system reduces the flow of solid, particulate organic matter, and nitrogen compounds into the environment and significantly increases the efficiency of water, when compared with a traditional culture system.

With this culture system, it is possible to recover up to 31.6% of the total nitrogen entering the pond and produce more than 4,000 kg ha^{-1} of shrimp.

The production system of interconnected ponds is technically feasible, and it also can incorporate innovations such as the use of bioreactors to increase consortia of heterotrophic microorganisms and other beneficial bacteria that help to improve the ecoefficiency of shrimp farming.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Integrated Evaluation of Urban Water Bodies for Pollution Abatement Based on Fuzzy Multicriteria Decision Approach

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Today's ecology is erected with miscellaneous framework. However, numerous sources deteriorate it, such as urban rivers that directly cause the environmental pollution. For chemical pollution abatement from urban water bodies, many techniques were introduced to rehabilitate the water quality of these water bodies. In this research, Bacterial Technology (BT) was applied to urban rivers escalating the necessity to control the water pollution in different places (Xuxi River (XXU); Gankeng River (GKS); Xia Zhang River (XZY); Fenghu and Song Yang Rivers (FSR); Jiu Haogang River (JHH)) in China. For data analysis, the physiochemical parameters such as temperature, chemical oxygen demand (COD), dissolved oxygen (DO), total phosphorus (TP), and ammonia nitrogen (NH_3N) were determined before and after the treatment. Multicriteria Decision Making (MCDM) method was used for relative significance of different water quality on each station, based on fuzzy analytical hierarchy process (FAHP). The overall results revealed that the pollution is exceeding at "JHH" due to the limit of "COD" as critical water quality parameter and after treatment, an abrupt recovery of the rivers compared with the average improved efficiency of nutrients was 79%, 74%, 68%, and 70% of COD, DO, TP, and NH_3N, respectively. The color of the river's water changed to its original form and aquatic living organism appeared with clear effluents from them.

1. Introduction

Massive ecosystem wide effects have been associated with their broad proliferation and toxin production [1]. The urban rivers or streams have always been the recipient of sewage water from various sources that have different kinds of the domestic, agricultural, and industrial foreign particles [2]. The odors released from these water bodies are offensive for environmental pollution; the release of odors has been often unavoidable due to its natural phenomena [3]. The municipal sewage is the mixture of various organic matters, and the decomposition of these matters produces harmful gases that in fact deteriorate the environment and logically created the infection diseases [4]. The inherent expectation is that villagers under this sewer situation have been leaving their houses because they face polluted potable water and skin and waterborne infectious diseases, and therefore urban environment situation has become more alarming [5, 6]. Municipal wastewater is the main cause of environmental impact, if it is directly discharged into urban water bodies or rivers without any sanitation preliminary treatment. Moreover, due to an increase in the shortage of clean water, there is a need for convenient management of accessible water resources.

To control environmental pollution would be a huge challenge for the planner and policy maker, due to treatment cost as well as the unbridled population acceleration. Here we need solution that efficiently resolves these critical pollution problems and could be used to rehabilitate the existing systems. In the last decades, some conventional technologies and methods have been developed and applied. To begin the application of reaeration (traditional technology) as adopting a series of weirs [2, 7], moving the wastage discharges place and local oxygenator were used for pumping air into the water body for wastewater treatment [8, 9]. The application of Multifunctional Constructed Wetland has been used for the treatment of wastewater and the purification of river water

[10–12]. Nitrogen and some other nutrient wastes were the vital source of pollution problems in China and unable to restore system through these conventional techniques. So, the anaerobic ammonia oxidation researches tend to move into full-scale treatment plant. Till now at least 5 genera and 13 species have been identified using culture-independent-molecular techniques [13]. Within the last decades some specialized reactor systems such as sequencing batch reactor, rotating biological contactor, trickling filter, UBF reactor, granular sludge bed reactor, and membrane bioreactor have been introduced in both laboratory and full scale to obtain high removal rate, and finally we noticed that these reactors played an important role in securing high rate performance for the product of nitrogen removal. Nitrogen pollution causes serious environmental problems and it not only threatens the sustainable development of fisheries, agriculture, tourism, and so forth, but also is harmful to the living environment of human beings. The amounts of phosphorus and ammonia nitrogen in domestic wastewater have been noticed from 10 to 17 and 30 to 50 mg/L, respectively [14].

Ecofriendly and sustainable environmental demand is the hot and impressive topic due to public, economic, and legislation pressure. The best selection in complex framework is based on the sustainability of nutrient removal, biodegradation of suspended particles, and removal efficiency of the system. In this situation, the major preference is to adopt any system that is more reliable for energy consumptions, conversion of chemicals into biomass, complex infrastructure, and the repairing or maintenance cost of the system. Bacterial Technology (BT) provides a plenty of opportunities for effectively treating these issues [2]. BT is an application of bioremediation that uses microorganism metabolism to remove nutrients from the water bodies and regenerate up to the original condition [2, 6, 9], and its operational cost is relatively low [15], which generally have a high public interest. Due to its smart application, it is popular in the research area of environmental sciences and engineering. Temperature is the major concern that is directly effective in the process of the degradation of the substances [9].

Due to these considerations, we can adopt the new technique as BT with complete confidence. Their bacteria are usually hired to vitiate pollutants or nutrients into simple or nontoxic entity and produce suitable effluents [16]. This technology has reassuring advantages compared to other traditional techniques as already discussed. BT has been successfully implemented to recover the polluted lakes [17], restore polluted rivers, and assimilate effluent of wastewater treatment plant [9]. To control the urban river pollution, BT has been employed in different places in China, that is, for treating the polluted urban water bodies [2, 15]. It was determined to be successful with reliable results in boosting up the recovery processes of all water bodies compared to the other traditional technologies. It extends for the rehabilitation of polluted lakes, rivers, and streams and is also reliable for the requirement of the wastewater effluent standards without constructing massive structures as compared to the other conventional methods [15, 16].

In the past, the water quality index approach was considered as the best tool to determine the water quality of the water bodies [17, 18]. Numerous researches represent the integrated uncertainty in evaluating the water quality. Some of these are under the base on fuzzy impartial optimization [19, 20] and MCDM problems using AHP [21]. Further, a few years ago, AHP and FAHP were acquiring popularity in the hydraulics and environmental engineering fields [22]. Srdjevic and Medeiros [23] have used FAHP for the management plans and Singh et al. [19] have used FAHP to determine the water quality of the Yamuna River that is tributary of Ganga. In this research, the urban rivers pollution abatement is being extensively determined at five various places, such as Xuxi River, Wuxi City; Gankeng River, Shenzhen City; Xia Zhang River, Yixing City; Fenghu and Song Yang Rivers, Ruian City; Jiu Haogang River, Hangzhou City. A location-wise variation of the water quality parameters (temperature, DO, COD, TP, and NH_3N) was determined before and after the treatment of the BT. For the relative significance of water quality parameters, AHP has been applied in the selected sites. In addition, FAHP is developed for the present research to determine the original status of water quality on each urban river based on MCDM framework. For current study, the data matrix is very complicated, because the spatial and temporal parameters vary from site to site. It is not possible with simple AHP to evaluate the pollution status on each station of each site. Therefore, FAHP is the best technique which can help to determine the water quality parameters values as compared to the other techniques. In this paper we briefly discuss practical implementation of BT on urban polluted rivers and argue with MCDM based on FAHP that BT is simple, affordable, and sustainable for restoring polluted water bodies.

2. Materials and Methods

2.1. Study Area and Samples Collection. Xuxi River (XXR) is situated in Wuxi City, Chang Nan District of China, geographically as (31°56.29′N and 120°28.14′E). Its upper stream starts from the Jing-Hang main canal and travels towards the ancient small canal. The selected river length for the experiment is 1360 m with 4.5 m of upstream surface average width and about the average depth of 1.4 m. River is under north subtropical humid zone and is marked by muddy sediments. This zone is facing four distinct seasons with the phenomenon of climatic influence circulation. Fenghu (FH) and Song Yong (SY) Rivers were selected, FH River is placed on the tail of the SY River, and both of them are situated in Wenzhou (Rui'an) City (120°39.13′E and 27°46.49′N), Zhejiang Province, China. Most of the Wenzhou area is placed under the typhoon zone, and the FH River is taking water from Wenruitang and Liangmian Rivers. The SY River is starting from cave bridge and falls directly into the FH River. The SY River length is about 280 m with the average breadth 5–18 m and 1–3 m water depth, and FH river length is about 740 m with the average breadth 6–15 m and 1 m water depth. For monitoring and sample collection of the experiment, the selected reaches of both rivers were divided into six points. The appearance of the river water color was blackish or greenish, and bubbles were blowing on the surface of water. These rivers are situated under the commercial and

Table 1: Water quality parameters before BT and Chinese National Standard.

Sampling time		Monitoring project						
		Water temperature ˚C	pH	DO mg/L	COD mg/L	TP mg/L	TN mg/L	NH_3N mg/L
National standard GB3838-2002		Class V index	6–9	2.00	15.00	0.0	2.00	2.00
A	14:40	16.1	7.5	2.5	10.90	0.96	14.80	10.60
	River water quality class		—	V	V	Inferior V	Inferior V	Inferior V
B	16:00	27.2	8.77	2.81	12.10	0.82	14.90	11.20
	River water quality class		—	V	V	Inferior V	Inferior V	Inferior V

* A, B represent two criteria based on pH and temperature value.

highly polluted area, and almost 2000 m^3 sewage water enters into them [2]. Therefore, the average depth of sediments is 0.1 m and the river's water quality was unsuitable for any purpose.

The remaining sites of Gankeng River (22˚33.24′N and 114˚34.63′E), Xia Zhang River (31˚26.49′N and 119˚49.13′E), and Jiu Haogang River (30˚18.59′N and 120˚09.07′E) are placed in Shenzhen City, Yixing City, and Hangzhou City of China. The averaged physiographic conditions of these rivers are the same as the above rivers. On the basis of Chinese surface water quality standard, the rank or class of water quality in the source section was determined to be grade V (Table 1). Class V shows the worst (poor) by the Chinese National Standard board and least water quality standard. For any purposes, this water quality of the river is extremely unsuitable. Hence, these sites selected for small urban rivers belong to the worst Class V category by the Chinese National Standard (CNS) board.

2.2. Bacterial Implementation. Bacterial Technology (BT) is applied in a simple way, and its procedure is held under three kinds of material as Bacterial Clusterization (BC), Nature Liquid, and Biological Filter Media (4 : 3 : 3). BC is an important material that has a mixture of three types of ingredients as beneficial bacteria (bacilli, *Bacteroides*, brown-rot spindle, and Lactobacillales, denitrifying with 6 : 4 : 3 : 4 : 3), mix medium (catalyst process as glucose, sucrose, cellulose liquid, yeast cream, liquorice root, magnesium sulfate, potassium hydride, mannitol, tartaric acid (Na; K), folic acid, and ammonium nitrate), and water [24]. The mixing ratio represents that it is harmless and has no any adverse effects. Nature Liquid (NL) is the mixture of trace element, multiple enzymes, humic acid, amino acid, and vitamins and composition of each adequate substance on judgment. Biological Filter Media are used on a domestic level as the gap string filter media.

By implementation of BT on site, the bacterial amount as BC is added to the selected points of each river as shown in Figure 1 (example on XXU site). As the bacterial agent is used to effectively work under relatively constant and slow flow of velocity, an artificial weir is installed at the end of the river reach which is the small wood bridge to stop effluent. It was technically built at about 50 cm high above the water surface level in order to extend the hydraulic retention time. This experiment was conducted from May 31 to July 31. To employ BT operation, the implementation procedure could vary based on the physical condition of the site. However, the method of adding beneficial bacteria directly to the polluted water body has proven to achieve desirable results for restoration programs. The addition of beneficial bacteria to polluted river is usually termed the Bacterial Technology.

2.3. Samples Collection Procedure. The sampling network was managed to cover the complete range along the inlet and outlet points of the rivers and determined the dominant point sources that have an impact on the water quality. Both of the sites are located under the area of population and industrialization, so the samples were collected from various depths (0.5 ft and >1.5 ft), at each monitoring station. The samples were collected from 8:30 AM to 4:30 PM during the period of experiment and 5 to 8 times in each month. To evaluate the water quality, the samples were kept in polyethylene bottles and stored in insulated ice cooler that were delivered to the laboratory on the same day. All the samples were saved at 4˚C until the analysis and processing.

3. Numerical Calculations for Data Treatment

All mathematical and statistical calculation was analyzed by using Excel 2007 and MATLAB Fuzzy Logic Function. There have been various methods on Multiattribute Decision Making (MADM) and the most useful is AHP which especially is based on pairwise comparisons on a ratio scale [25]. According to some AHP limitations the fuzzy modification of AHP (FAHP) was then posed that is the subject of this study.

3.1. Analytical Hierarchy Process (AHP). AHP is an MCDM method that provides the hierarchical framework to illustrate the concern objective and developed the scale of priority based on the application judgment [25]. The AHP operation belongs to six essential steps [26] as shown in Figure 2.

3.1.1. Define the Unstructured Problem. We define the concern objectives and consequence of the unstructured problem and the recognition of the specific characteristics.

3.1.2. Developing the AHP Hierarchy. The AHP is based on the decision disintegration of the hierarchy unstructured

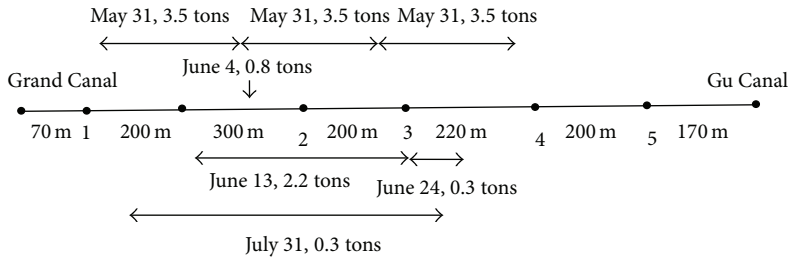


Figure 1: Schematic diagram of the Xuxi River and sampling points during BT.

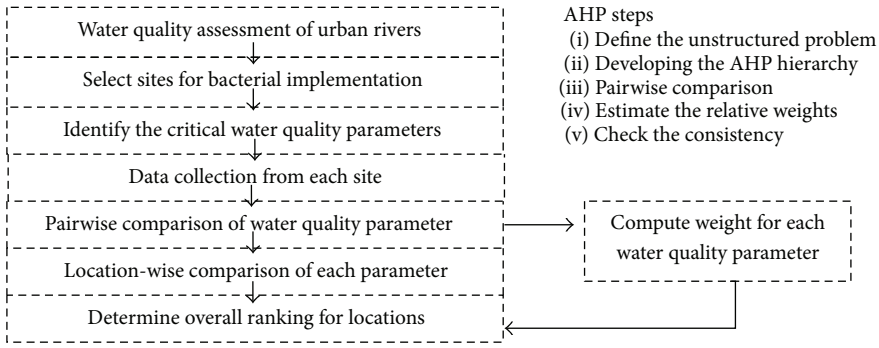


Figure 2: AHP for judgment.

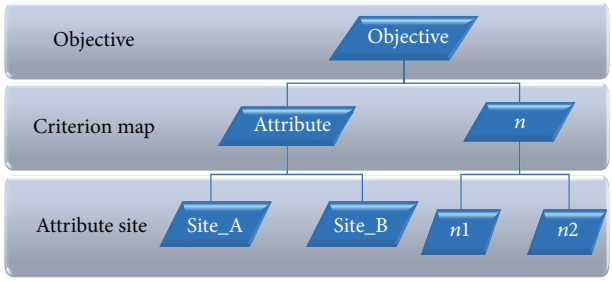


Figure 3: Hierarchical structure of decision problem.

problem that resides in the decision problem of the most important element [27]. The complicated task is decomposed into a hierarchical structure (Figure 3) with the elements of decision.

3.1.3. Pairwise Comparison. For pairwise comparison matrices of each element of the hierarchy structure are compared as follows:

$$A = \begin{bmatrix} 1 & \frac{w_1}{w_2} & \cdots & \frac{w_1}{w_n} \\ \frac{w_2}{w_1} & 1 & \cdots & \frac{w_2}{w_n} \\ \vdots & \vdots & \vdots & \vdots \\ \frac{w_n}{w_1} & \frac{w_n}{w_2} & \cdots & 1 \end{bmatrix}, \quad (1)$$

Table 2: Scales for pairwise comparison [25].

1	Equal importance
3	Moderate importance
5	Strong importance
7	Very strong importance
9	Extreme importance
2, 4, 6, 8	Intermediate values between adjacent scale values

where A is matrix of pairwise comparison, w_1 is element weight 1, w_2 is element weight 2, and w_n is element weight n.

For the decision of the relative significance between hierarchy elements in matrix A, a linguistic scale is employed for the values to be rated from 1 to 9 (Table 2).

3.1.4. Estimate the Relative Weights. The relative weights of elements in each pairwise comparison matrix are determined by some methods like eigenvalue method. The relative weights (W) of matrix A are determined as

$$(A - \lambda \max I) \times \omega = 0, \quad (2)$$

where λmax is matrix A as biggest eigenvalue and I is unit matrix.

3.1.5. Check the Consistency. The matrices consistency property is determined to ensure that the judgments of decision makers either are consistent or need more iterations.

Table 3: Random inconsistency indices [25].

Number of criteria	1	2	3	4	5	6	7	8	9	10
RI	0	0	0.58	0.9	1.12	1.24	1.32	1.41	1.45	1.49

Consistency Index (CI) can be measured from the following equation:

$$\mathrm{CI} = \frac{\lambda \max - n}{n - 1}. \tag{3}$$

The reciprocal matrix is generated from the random Consistency Index that would be known as the random index (RI). A sample size of 100 was used to generate the average RI for the matrices of order of 1–15 [28]. The Saaty matrices represent the RI (Table 3) that can be seen in the order of 1–10 [25]. At last, if CR $<$ 0.1, the judgments from the above procedure are consistent and the derived element's weights can be considered for the further analysis. The formulation of CR is

$$\mathrm{CR} = \frac{\mathrm{CI}}{\mathrm{RI}}. \tag{4}$$

3.1.6. Obtain the Overall Rating. At the end, the relative decisions of element weights are compiled to gain the whole alternatives rating as follows:

$$w_i{}^s = \sum_{j=1}^{j=m} w_{ij}{}^s w_j{}^a \quad i = 1, \ldots, n, \tag{5}$$

where $w_i{}^s$ is total weight of "i" site, $w_{ij}{}^s$ is weight of alternative i associated with attribute j, $w_j{}^a$ is weight of attribute j, n is number of sites, and m is number of attributes.

3.2. Fuzzy Analytical Hierarchy Process (FAHP). Despite the recognition of AHP often this method is censured to sufficiently handle its failure for the imprecision and latent uncertainty associated with the grading of the decision maker's perception of exact values [29]. Fuzzy AHP as an extension of AHP investigate to be more efficient tool in the water management decision problems [30, 31]. Since vagueness and fuzziness are ordinary characteristics in a number of decisions, a FAHP method should be able to indulge ambiguity or vagueness [32]. In FAHP, the eigenvector method is applied to simulate the reciprocal matrix and to evaluate the importance and alternative performance across the criteria. The additive weighting method is applied for the determination of the use of alternative across criteria. When complex multifeatures are considered for decision making problems, FAHP has skill of capturing an uncertainty of human assessment [33]. This procedure is applied to determine the crisp judgments into fuzzy judgments [34]. This classic fuzzy set theory allowed $[0, 1]$ range of real numbers to operate the participation functions. The major fuzziness function is the individuals grouping elements into classes without clearly defining the boundaries [35]. The uncertainty judgment of comparison

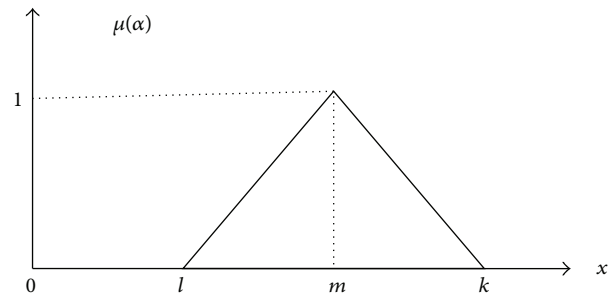


Figure 4: Fuzzy triangular Number.

can be indicated by the fuzzy number. A fuzzy number of the triangle is defined by three real numbers (Figure 4) which belong to special class, expressed as (x, b, k). The fuzzy numbers of the triangle are determined as follows:

$$\mu(\alpha) = \begin{cases} (x - l)(m - l), & l \le x \le m, \\ (k - x)(k - m), & m \le x \le k, \\ 0, & \text{otherwise.} \end{cases} \tag{6}$$

In order to compose pairwise alternatives comparison under each criterion or benchmark, a triangular fuzzy comparison matrix is indicated as follows:

$$\begin{bmatrix} (1,1,1) & (l_{12}, m_{12}, k_{12}) & \cdots & (l_{1n}, m_{1n}, k_{1n}) \\ (l_{21}, m_{21}, k_{21}) & (1,1,1) & \cdots & (l_{2n}, m_{2n}, k_{2n}) \\ \vdots & \vdots & \vdots & \vdots \\ (l_{n1}, m_{n1}, k_{n1}) & (l_{n2}, m_{n2}, k_{n2}) & \cdots & (1,1,1) \end{bmatrix}, \tag{7}$$

where $\tilde{\alpha}_{ij} = (l_{ij}, m_{ij}, k_{ij})$; $\tilde{\alpha}_{ij}{}^{-1} = (l_{ji}, m_{ji}, k_{ji})$, for $i, j = 1, \ldots, n$ and $i \neq j$.

Total alternatives preferences and weights can be acquired from different method. In this study, these two approaches or techniques will be posed in renewal.

3.2.1. Fuzzy Logic Process for Experiment. A fuzzy analytical hierarchy process (FAHP) has been developed to evaluate the status of water quality at the selected stations along each river under a Multicriteria Decision Making framework. A decision support mechanism has been introduced to select and prioritize stations, with specific reference to the universal principle as written below:

(a) Moving water tends to contain more DO than stagnant water.

(b) The DO concentration is inversely proportional to temperature.

(c) Health of water quality is based on the requirement of organism that lives in it.

(d) pH scale verifies the acidity and alkalinity of wastewater.

(e) The overenrichment of a body of water by nutrients like nitrates and phosphates is cause of eutrophication.

Table 4: Water quality data of experiment.

Parameters	GB2828-2002 (CNS)		XXU	GKS	XZY	FSR	JHH
Tem. (°C)	15–30	Mean	24.7	25.5	16.5	16.2	28.7
		Std. deviation	0.29	0.46	0.32	0.34	0.35
		Minimum	19.7	24.6	16.2	15.6	27.9
		Maximum	27.8	25.8	17.1	16.8	29.4
DO (mg/L)	2	Mean	1.69	1.79	1.83	1.14	3.4
		Std. deviation	1.08	0.9	1.9	0.7	1.38
		Minimum	0.4	0.4	0.5	0.8	0.7
		Maximum	3.3	2.7	4.8	1.8	4.5
COD (mg/L)	15	Mean	14.5	16.4	59.7	43.5	59.01
		Std. deviation	6.8	2.45	17.5	23.5	35.2
		Minimum	5.4	4.1	29	24.7	24
		Maximum	17.7	18.1	67.2	86	139
NH_3N (mg/L)	2	Mean	15.21	16.87	22.49	13.59	21.44
		Std. deviation	9.77	10.23	9.99	7.5	10.44
		Minimum	9.42	8.01	12.04	0.87	8.76
		Maximum	27.3	26.9	29	15.7	26.7
TP (mg/L)	0	Mean	0.91	1.2	0.83	1.3	1.9
		Std. deviation	0.57	0.5	0.49	0.47	0.42
		Minimum	0.14	0.18	0.07	0.5	0.7
		Maximum	1.86	1.57	1.2	1.7	2.8

The various water quality parameters have been considered as criteria to evaluate water quality status at a given station of each project site. Pairwise comparisons of the criteria and the stations have been performed to assess water quality using linguistic variables.

4. Result and Discussion

The selected urban rivers are situated under the appalling environment and the river's conditions were awful before the operation of BT. The huge amount of sewage was loaded directly and entered into these rivers. In addition, it is observed that there was not any preliminary facility to control or dump the domestic sewage. Therefore, the sewage is partially or directly a part of the urban river without any pretreatment. Under this alarming situation, the river's color was changed into greenish representing the thick oil floats and debris. Therefore, in this sewer condition, any living organism in the river water could not exist.

BT has been applied and water samples from the selected points were collected before and after the treatment of the experiment. The physiochemical parameters were collected on the specific monitoring points on every site. The range, mean, and standard values of each parameter are in Table 4; after determining the values, we compared the improved efficiency of nutrients before and after bacterial action which was 79%, 74%, 68%, and 70% of DO, COD, TP, and NH_3N, respectively. From the results, the DO was the most critical parameter for aquatic life of rivers that have maximum efficiency. To protect the environmental pollution, we determined that TP and COD efficiency also have favorable results. For more consideration, the color and algal from every site are also recovered as shown in Figures 5(a)–5(e).

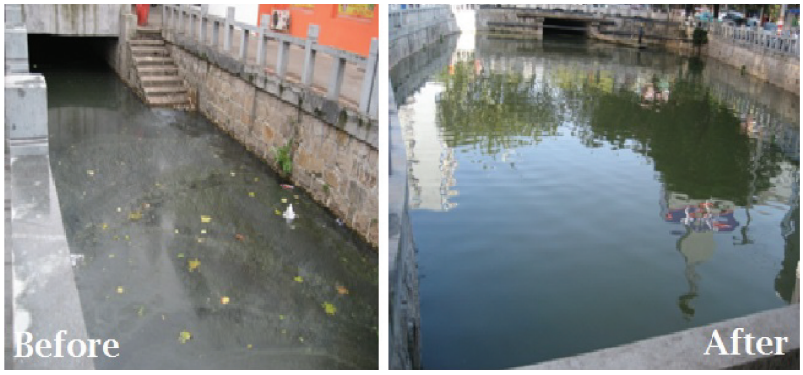
Table 5: Pairwise comparison matrix of the various water quality parameters.

Parameters	DO	COD	TP	NH_3N	Temperature
DO	1	1/3	1	1/1.5	1/5
COD	3	1	2	1/1.5	1
TP	1	1/2	1	1/2	1
NH_3N	1.5	1/2	2	1	1/1.5
Temperature	5	1	1.5	1.5	1

4.1. Numerical Evaluation of the Experiment. The fundamental statistics of these restoration experiments are based on 2760 total water samples (23 sampling stations × 4 sampling frequencies × 5 replications × 6 months) and are summarized in Table 4 which represents the range, mean, and standard deviation of the results for each parameter. The data were collected during 6 months and each station of the site was monitored with spatial as well as temporal variation.

FAHP developed a selection support tool that describes the pairwise priority of the station with the particular beneficial reference, such as domestic, aquatic status, irrigation, and recreational and industrial enterprises. The pairwise comparison matrix was formulated due to the variations and the complicity of the water quality parameters on each site, and the comparisons were accomplished based on the convincing of engineering results and each water quality parameter of all sites was formulated in Table 5.

For the evaluation of the relative weights, the comparisons of all five locations with each parameter (Tables 6–10) were measured with the ambition to determine the actual status of the water quality improvement from before and after the BT operation.



(a)

(b)

(c)

(d)

Figure 5: Continued.

(e)

Figure 5: (a) Contrast diagram of Fenghu and Song Yang Rivers. (b) Contrast diagram of Xia Zhang River. (c) Contrast diagram of Xuxi River. (d) Contrast diagram of Jin Haogang River. (e) Contrast diagram of Gankeng River.

Table 6: Location-wise comparison matrix for temperature.

Site	XXU	GKS	XZY	FSR	JHH
XXU	1.00	0.33	0.67	2.00	0.29
GKS	3.00	1.00	1.49	4.00	0.67
XZY	1.50	0.67	1.00	2.00	1.00
FSR	0.50	0.25	0.50	1.00	0.33
JHH	3.50	1.50	3.00	3.00	1.00

Table 7: Location-wise comparison matrix for DO.

Site	XXU	GKS	XZY	FSR	JHH
XXU	1.00	1.50	0.33	0.29	0.57
GKS	0.67	1.00	0.29	0.50	1.00
XZY	3.00	3.50	1.00	4.00	1.00
FSR	3.50	2.00	0.25	1.00	3.00
JHH	1.75	1.00	0.33	0.33	1.00

Table 8: Location-wise comparison matrix for COD.

Site	XXU	GKS	XZY	FSR	JHH
XXU	1.00	2.00	4.00	0.80	4.00
GKS	0.50	1.00	0.33	0.59	4.00
XZY	0.25	3.00	1.00	0.67	1.00
FSR	1.25	1.70	1.50	1.00	2.00
JHH	0.25	0.25	0.25	0.50	1.00

Table 9: Location-wise comparison matrix for NH_3N.

Site	XXU	GKS	XZY	FSR	JHH
XXU	1.00	0.80	0.40	1.33	0.67
GKS	1.25	1.00	0.67	2.00	0.33
XZY	2.50	1.50	1.00	9.09	1.00
FSR	0.75	0.50	0.11	1.00	0.40
JHH	1.50	3.00	0.40	2.50	1.00

Table 10: Location-wise comparison matrix for TP.

Site	XXU	GKS	XZY	FSR	JHH
XXU	1.00	4.00	1.49	0.67	0.20
GKS	0.25	1.00	0.67	0.29	0.33
XZY	0.67	1.50	1.00	0.67	1.00
FSR	1.50	3.50	1.50	1.00	0.67
JHH	5.00	3.00	0.25	1.50	1.00

Table 11: Criteria ranking of water quality parameters.

Parameters	Scores	Ranking
Temperature (°C)	0.305	1
COD (mg/L)	0.277	2
DO (mg/L)	0.204	3
TP (mg/L)	0.116	4
NH_3N (mg/L)	0.097	5

The main concern in this present contribution is to explain the actual BT function to mitigate the pollution from urban water bodies. AHP based on FAHP results are applied to the ranking of the water quality parameters (Table 11) as well as the location ranking with an overall inconsistency of 0.076 (Table 12). The location-wise variations score is illustrated in Figure 6, which represent the high ranking of the JHH site as compared to the others.

After the evaluation of the current status from the results, the actual pollution status of each site after the BT operation is revealed. Figure 6 displays the summary of the results with the overall inconsistency of 0.076, less than 10%. Its describe the COD value is extremely high (up to 139 mg/L) at JHH site which display the major cause of pollution. Besides, the river is placed in the industrial area. Similarly the value of TP is also high because, in the middle, a cement factory is working and the wastewater directly enters into the river. Instead of all, for the evaluation of BT, in the beginning of operation, there was an appalling condition as blackish water and odors that made part of the pollution. When we applied BT operation, the polluted river was changed up to reliable condition without

Table 12: Criteria ranking of sites (overall inconsistency = 0.076).

Site	Scores	Ranking
JHH	0.310	1
FSR	0.241	2
XXU	0.191	3
XZY	0.175	4
GKS	0.083	5

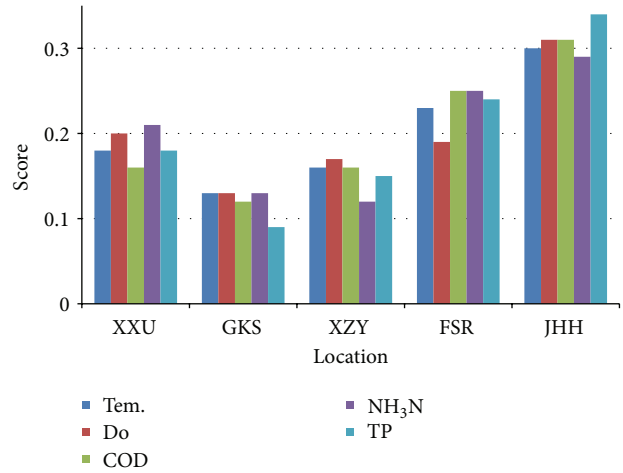


Figure 6: Ranking criteria of each water quality parameter in each location.

any odors. The river water color was also changed from blackish to its original form. BT restored the JHH River and it came back to its habitat environment. However, we can adopt these advanced technologies to rehabilitate our environment, but there is a need to manage or fix horrific point sources of pollution.

4.2. Cost Benefit Ratio Based on Conventional Technologies. Temperature is the major concern under the metabolism process of the bacteria, with higher temperature during the summer (20–30°C) and lower values in the winter season (10–18°C). The plus advantage of BT does not require destruction of an already built system. BT has no effect on the natural environment because it does not involve the use of chemicals. Therefore, it is helpful for friendly ecology. It is free from all other issues, as high construction and maintenance costs can be a huge burden to organization and policy makers.

In view of this, with revolutionary calculations, the adoption of BT has been concluded to be the most convenient approach for developing countries [15]. The cost to treat the tons of wastewater is about 241≈321$, where WWTPs are being built, under construction, or already built, and the operation cost is between 0.12 and 0.22$ per ton. According to this statement, for the municipal sewerage operation system needs to spend above $16 \times 10^7$$ on single attempt. If the pipe network of municipal administration is built, the amount of total cost will exceed $32 \times 10^7$$, and the operational cost is raised up to $4 \times 10^7$$ annually. Therefore, with the addition of bacteria to treat the sewerage wastewater, the one-off investment cost is only 65$ and this method is simple, easy to operate, and affordable. For the long term, BT maintenance and artificial dregs cannot be needed for 10 years in the future [36]. In addition, the existing sanitation systems are deteriorating due to many-imperfection care. So BT has ability to restore these systems due to its self-purification property. Similarly the maintenance cost of the sewerage system is unfavorable due to economic collapse. So we can prefer this technology due to its simplicity and low cost.

5. Conclusions

In order to rehabilitate the urbanized water bodies as lakes, rivers, and streams, BT is sustainable and reliable for public health with no maintenance and further general costs to minimize the traditional system. In this study, we demonstrate the interpretation of the water pollution problems of a complex dataset through MCDM techniques, because chemometric research enables us to discuss the similarities and dissimilarities along the observing stations among the variables that could not be clearly visible for assessment of the analytical data in a table. This research emphasized that the BT offers an ingenious and innovative solution for rehabilitation of the urban water bodies up to the suitable water quality. BT is efficient due to its simplicity, being economically affordable and reproducible on any scale of the operation; hence, it can provide tenable and long-term solution to the various water related pollution problems all over the world.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

The Regulation by Phenolic Compounds of Soil Organic Matter Dynamics under a Changing Environment

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Phenolics are the most abundant plant metabolites and are believed to decompose slowly in soils compared to other soil organic matter (SOM). Thus, they have often been considered as a slow carbon (C) pool in soil dynamics models. Here, however, we review changes in our concept about the turnover rate of phenolics and quantification of different types of phenolics in soils. Also, we synthesize current research on the degradation of phenolics and their regulatory effects on decomposition. Environmental changes, such as elevated CO_2, warming, nitrogen (N) deposition, and drought, could influence the production and form of phenolics, leading to a change in SOM dynamics, and thus we also review the fate of phenolics under environmental disturbances. Finally, we propose the use of phenolics as a tool to control rates of SOM decomposition to stabilize organic carbon in ecosystems. Further studies to clarify the role of phenolics in SOM dynamics should include improving quantification methods, elucidating the relationship between phenolics and soil microorganisms, and determining the interactive effects of combinations of environmental changes on the phenolics production and degradation and subsequent impact on SOM processing.

1. Introduction

Phenolics consist of more than one aromatic ring, bearing one or more hydroxyl functional groups. They originate from plant materials and industrial products/wastes, which enter the soil either as leachates or as particulate matter [1]. Once integrated into the soil, phenolics can control below-ground processes, including SOM decomposition [2–4] and nutrient cycling [5, 6]. Recently, Freeman et al. [7] have suggested that modification of phenolics in peatland has a potential as a geoengineering tool to capture C in terrestrial ecosystems.

In spite of theses multitude of studies, however, controversies remain on how phenolics decompose in soils, how they modify the rate of SOM decomposition, and how current environmental changes will influence the fate of phenolics in soils. Given that phenolics represent one of the most abundant components in soils [8, 9] and that they affect the cycling of key nutrients to plants and soil microorganism [1, 9], it is indispensable to investigate the mechanisms by which phenolics influence decomposition biotically and abiotically and the degree to which these mechanisms will vary in response to environmental changes. This review presents current knowledge about phenolics and their role in decomposition under various environmental changes and proposes areas of future research. This review covers the following six areas: (1) various structures and forms of phenolics in soils; (2) how to extract and measure phenolics in soil samples; (3) biodegradation of phenolics; (4) effects of phenolics on SOM decomposition; (5) effects of environmental changes, such as elevated CO_2, warming, N deposition, and drought, on phenolics and decomposition; and (6) suggestions for future phenolics studies.

2. Structure and Form in Soils

Naturally, phenolic compounds are widely distributed throughout the plant kingdom, constituting up to 60% of plant dry mass [10]. Due to its loose definition (presence of at least one aromatic ring and hydroxyl group), more than 8,000 compounds have been classified as phenolics to date [11], encompassing simple, low molecular compounds to complex,

Table 1: Methods to quantify phenolic compounds.

Assay	Types of phenolics	Description	Reference
Folin-Ciocalteu assay	Total phenolic acids	An assay based on electron transfer (ET) in which oxidation of phenolics by Folin-Cioalteu reagent gives a colored product at 750 nm	[12]
CuO oxidation-GC	Lignin-derived phenolics	A method in which oxidation of lignin by cupric oxide yields single-ring phenol compounds (vanillyl-, syringyl-, and p-coumaryl units), followed by gas chromatography Also, the acid to aldehyde ratio can be used to estimate the state of decomposition of lignin	[13]
HPLC	Individual	A separation technique in which a mixture of phenolics produces different retention times depending on their affinity to the stationary phase	[14]

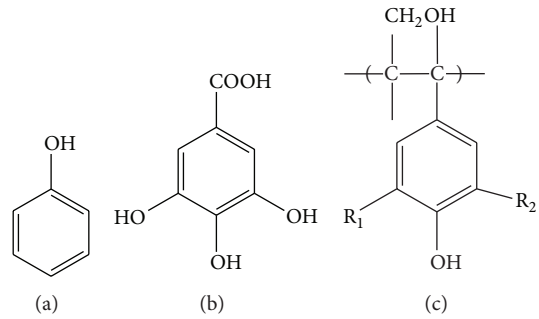

Figure 1: Chemical structures of several phenolics: phenol (a), the simplest structure of phenolic compound, phenolic acid (gallic acid) (b), and tannin (c).

highly polymerized compounds. Often, the number of aromatic rings and chemical structure are used to classify phenolics (Figure 1). For example, phenol, the simplest form of phenolics, has one aromatic ring with no extra carbon and belongs to class simple phenols. Class phenolic acids have a basic structure of C6-C1, including gallic acid, vanillic acid, and syringic acid. Lignin, one of the most common compounds in plants, is categorized as class lignins, exhibiting multiple combination of C6-C3 structure.

Phenolics in soils can exist as (1) a dissolved form, which moves freely in the soil solution, (2) a sorbed form, which reversibly binds to the soil particle or proteins, and (3) a polymerized form, consisting of humic substances (Figure 2). As many phenolics including phenolic acids and tannins are water soluble, they remain in solution between soil particles [15]. Reversible sorption of phenolics by soils occurs through hydrophobic, hydrogen, and ionic bond [8]. Humic substances, a stable polymer in soils, are generated by a polymerization of phenolics with other phenolics or soil organic matter [9].

Recent studies suggest that the form of phenolics, not their chemical structure, can influence their fate in soils [16–19], raising a question on conventional classification of phenolics into a slow, recalcitrant pool in C dynamics climate model [20]. For example, dissolved phenolics may have higher chance than sorbed or polymerized ones to encounter microorganisms in soil solution, allowing them to be processed quickly into simple, assimilable forms. In contrast, physically and chemically protected phenolics can persist longer than dissolved forms, providing feedbacks to SOM-decomposing microorganisms via changing soil pH, nutrient availability, and enzyme activities. Thus, caution is required to investigate the role of phenolics in SOM decomposition.

3. Extraction and Quantification of Phenolics

A variety of methods of extraction and quantification of phenolic compounds in soils have been established (Table 1). Solvents such as water, acetone, methanol, and citrate are widely used to extract phenolics. Blum [21] reported that soil samples extracted by water and citrate were suitable for estimating both free phenolic acids and sorbed phenolics. Arditsoglou and Voutsa [22] showed that acetone has higher extraction efficiency than methanol in aqueous samples. In contrast, Mukhopadhyay et al. [23] revealed that a mixture of methanol and water (6 : 4, v/v) was best to extract total phenolics and individual phenolic acids from black cohosh.

As the amount of phenolic compounds in soils can vary, the Folin-Ciocalteu assay is commonly used to determine the total amount of phenolic acids [24–26]. This assay is relatively simple compared to the CuO oxidation and the HPLC method. Thoss et al. [27] have compared 5 different methods to measure phenolic content in various freshwater samples. They concluded that a different pattern for each site originated from reactivity of phenolic materials and that Folin-Ciocalteu assay is the most appropriate for measurement of total phenolics. However, Ohno and First [28] pinpointed the limitations of the Folin-Ciocalteu assay that it is suited only for samples extracted by water, and interference by organic matter, such as sugars and aromatic amines, makes it impossible to precisely measure the amount of phenolic acids in citrate-extracted soils. In addition, the Folin-Ciocalteu assay was criticized for its low sensitivity [21].

Prior et al. [36] suggested correcting for nonphenolic compounds by using gallic acid as a reference for standardization. For quantification of highly polymerized lignin, gas chromatography (GC) followed by CuO oxidation is

Table 2: Extracellular enzymes involved in phenolics degradation in soils.

Enzyme	Microorganism	Optimum condition		Reference
		pH	Temperature	
Lignin peroxidase	*Phanerochaete chrysosporium*	2.5		[29]
	Phanerochaete chrysosporium	4.2	34	[30]
Manganese peroxidase	*Phanerochaete chrysosporium*	4.5	32	[31] [30]
	Phanerochaete sordida	4.5~5.0		[32]
Laccase	*Trametes versicolor*	2.0		[29]
	Basidiomycete PM1	4.5	80	[33]
	Pycnoporus sanguineus	3~5	55	[34]
Phenol oxidase	*Termitomyces albuminosus*	2.3		[35]

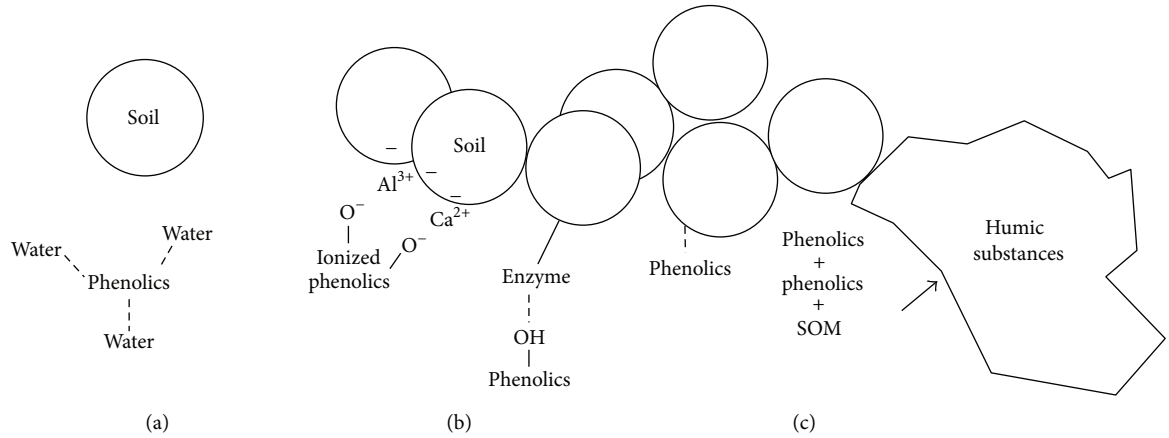

Figure 2: Various forms of phenolic compounds in soils. A dissolved form (a) where phenolics make multiple hydrogen bonds with water molecules surrounding them. A sorbed form (b) where phenolics are absorbed in soils and may detach from them reversibly through ionic, hydrogen, and hydrophobic bonds. A polymerized form (c) where phenolics consist of humic substances connected with other soil organic matter.

employed [37, 38]. CuO oxidation has the potential to be a powerful tool to estimate lignin content in soils as well as the degree of lignin decomposition [39]. Even though GC yields a high sensitivity, the low volatility of simple phenolic compounds requires a derivatization step, resulting in longer sample preparation [40]. Over recent decades, analysis of phenolics has been conducted via high performance liquid chromatography (HPLC) [41–44]. However, wide use of HPLC in ecological studies has been restricted by high cost and complicated process of operation.

4. Degradation of Phenolics

In soils, phenolics are mainly degraded by fungi (e.g., Basidiomycetes and Ascomycetes) and bacteria (e.g., *Pseudomonas*). These microorganisms release extracellular enzymes into soils that break down phenolic compounds (Table 2). Phenolics-degrading enzymes are often named as phenol oxidase or peroxidase, according to their electron acceptor [45]. Both enzymes cause nonspecific oxidation of phenolic compounds, consuming oxygen and hydrogen peroxide as an electron acceptor, respectively.

Environmental factors, such as soil pH, temperature, oxygen, and substrate, can affect the degradation of phenolics. Contrary to the relatively low optimal pH of purified enzymes in laboratory conditions (Table 2), Sinsabaugh [45] found that there is a positive relationship between phenolics-degrading enzyme activities and soil pH across ecosystems. Likewise, Pind et al. [24] have reported that phenol oxidase activity increases as pH of peat soils increases. Regarding temperature, phenol oxidase showed no clear relationship [46–48] in the field conditions probably due to the interactive effecs of oxygen availability at different temperatures. However, purified phenol oxidase increased its decay of L-DOPA, a proxy of phenolics in lab conditions, at a temperatture of 5–25°C [49]. As phenol oxidase uses oxygen as an electron acceptor, its activity is proportional to oxygen concentration [24]. The relationship between the activity of phenol oxidase and phenolics concentration in natural ecosystem is not clear, as conflicting evidence is currently present. While some studies reported a positive relationship [2, 38, 50, 51], still others demonstrated contradictory results, reporting a negative or inverse relationship [25, 52–54] or no relationship [55, 56].

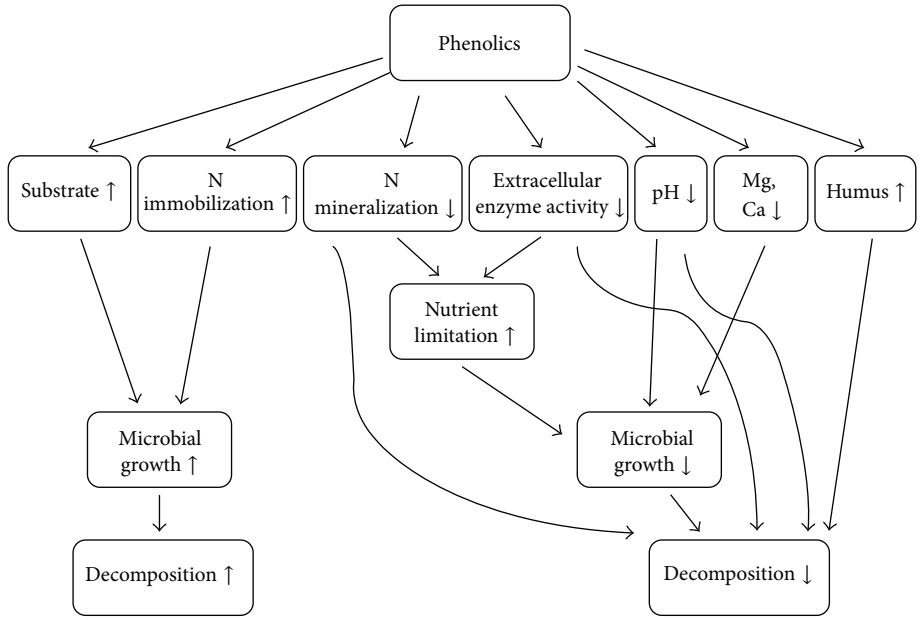

Figure 3: Effects of phenolics on the rate of soil organic matter decomposition.

Such diverse responses, however, should be interpreted with care. In case of soil systems with a large amount of phenolics such as peat matrix, higher phenol oxidase in soil results in higher phenolic content in pore water as a product of enzyme action on peat, resulting in a positive relationship between phenol oxidase and phenolics. However, if correlation analysis was conducted between phenol oxidase in soils and phenolics in soil matrix or soil extract in mineral soils such forest soils, a negative correlation has often been reported because here phenolics may represent an enzyme substrate rather than a product. Another possibility is the dual functions of phenol oxidase. For example, Burke and Cairney [57] pointed out that mycorrhizal laccases can mediate both in depolymerization and polymerization and that, without the knowledge of redox mediators for these enzymes, predicting the direction of phenolics processing may be difficult.

Degradation of phenolics is usually reported by several groups to be slower than the degradation of other SOM fractions. The litter bag experiment demonstrated that labile compounds in litter such as carbohydrates and proteins were preferentially decomposed over phenolic compounds [3, 58]. As such, phenolic concentrations have been useful in predicting the rate of litter degradation [59, 60]. Yet, as stated above in Section 2, phenolics can decompose fast in certain conditions. Soluble phenolics and tannins degraded to 36~50% of the initial content in the litter bag experiment [61, 62]. Degradation of lignin, determined by the acid to aldehyde ratioin CuO oxidation products (see Table 1), was dominant over degradation of other SOM in forest soils [63]. ^{14}C-labelling revealed that 56~68% of lignin from maize was transformed into CO_2 during 6 months of a laboratory incubation [64].

5. Effects of Phenolics on Other SOM Decomposition

Effects of phenolics on SOM decomposition have been studied directly (i.e., litter bag) or indirectly (i.e., microbial biomass, extracellular enzyme activity, and heterotrophic respiration). Generally, phenolics reduced the rate of litter/SOM decomposition [65, 66]. Moreover, phenolic acids released by *Sphagnum* in peatlands suppressed bacterial and fungal growth [67, 68]. Even low concentrations of phenolics in peat homogenates have been noted to inhibit the activity of β-glucosidase, phosphatase, sulphatase, chitinase, and xylosidase by 21, 15, 32, 18, and 14%, respectively [69]. In addition, dissolved organic matter containing phenolic compounds from peat samples decreased CO_2 production in anaerobic conditions [70]. Likewise, the rate of litter decomposition was shown to be inversely proportional to the phenolic content in litter [61].

As illustrated in Figure 3, the inhibition of decomposition by phenolics can occur via (1) formation of covalent bonds with proteins, decreasing N mineralization and enhancing N limitation to microorganisms [71], (2) oxidation of other phenolics, leading to humus formation [72], (3) suppression of microbial growth by lowering pH [73], (4) deprivation of metal ions by their high cation-exchange capacity [74], or (5) a formation of phenolic-enzyme complex, inactivating decomposition activity [75]. Yet, there are several studies beyond this simple negative relationship between phenolics and decomposition. Fierer et al. [76] found out that low molecular phenolic compounds and some tannins could serve as a labile substrate, promoting microbial biomass. Müller et al. [77] showed that lignin-derived phenolic compounds induced cellulase production, suggesting their

potential to enhance decomposition. Significant reduction in SOM content was also observed after phenolics were added [78]. In agreement with this finding, phenolic concentrations have been reported to be positively correlated to CO_2 release from soil [79] or litter [66].

Opposing reviews on the effect of phenolics on SOM decomposition argue further studies on the relationship between the forms and the roles of phenolics on decomposition and clearer terminology, as a wide range of molecules are defined as phenolics. In general, simple phenolics, such as phenolic acids, appear to increase decomposition, while complex phenolics decrease decomposition. Hoostal and Bouzat [80] showed that microbial extracellular enzyme activities were dependent on the source and composition of phenolics, rather than the absolute quantities of phenolics.

6. Effects of Environmental Changes on Phenolics

So far, several studies have aimed at elucidating how environmental changes such as elevated CO_2, warming, N deposition, and drought may affect phenolic production from plant tissues, subsequent degradation in soils, and SOM decomposition.

Elevated CO_2 usually increases phenolic concentrations in plants (Table 3). In field CO_2 enrichment experiments, phenolic compounds in plant tissues, such as leaves, needles, stems, and rhizomes, increased by 11–182% [81–84]. Elevated CO_2 can increase carbon supply and nutrient (e.g., N) stress in trees, resulting in decreased carbon demand. Such change is known to accelerate the accumulation of total nonstructural carbohydrates and the synthesis of carbon-based secondary or structural compounds [81]. Change in the concentration of phenolics from plant tissues may impart its effect on downstream processes including SOM decomposition. For example, Siegenthaler et al. [58] found that elevated CO_2 induced a production of phenolic-rich litters, resulting in declining SOM decomposition. Effects of elevated CO_2 on phenolic production in wetlands including peatlands have been extensively studied because wetlands are one of the key sources of DOC and phenolics to aquatic ecosystems. For example, elevated CO_2 increased DOC and phenolic leaching from wetlands [69, 85], which may decrease hydrolase activities [2]. However, some studies have reported a faster degradation of phenolics at elevated CO_2. After 559 days of litter bag incubation, lignin loss from Mongolian oak fine roots was 13% faster in the elevated CO_2 chamber than in the ambient chamber, which was attributed to a 10% increase in phenol oxidase activity compared to the control nontreated group [86]. Moreover, phenolic compounds in an ombrotrophic bog decreased by 15.4% at elevated CO_2 compared to control [87], suggesting that elevated CO_2 may accelerate phenolic degradation. It appears that elevated CO_2 often increases the total amount of carbon supplied to belowground microorganisms and may induce "priming" effects to accelerate the decomposition of old or recalcitrant organic matter. Norby et al. [88] studied the effects of elevated CO_2 on litter chemistry and decomposition rates in upland vegetation and demonstrated that elevated CO_2 does increase lignin content in leaf litter significantly, but there is no significant effect on decomposition rate. In summary, further investigation is warranted on the effects of increasing phenolics on decomposition because of the involvement of other factors such as vegetation types, ecosystem types, nutrient availability, and changes in other factors (e.g., temperature and water availability).

Rising temperature is expected to be accompanied by an increase in atmospheric CO_2 concentration. Few studies have measured the effect of warming on phenolic production and degradation, with an emphasis on whole organic matter decomposition. Unlike the rather unidirectional influences of elevated CO_2, warming has various effects on the production of phenolics (Table 3). Increases in temperature have led to both an increase [89] and a decrease [90] in phenolic production. Warmer conditions usually accelerate biochemical reactions and may result in lowering production of secondary metabolites because plant growth would be enhanced. In fact, Zvereva and Kozlov [91] have reported lower phenolic contents under warming conditions than control in their meta-analysis. However, interactive or simultaneous effects of elevated CO_2 and warming in relation to phenolics production have not been reported [83, 90] because two effects often negate each other [91].

N enrichment was studied in terms of atmospheric N deposition and fertilizer additions. Many studies suggest that phenolic concentrations are unchanged after N enrichment [92–94]. Extracellular enzymes, such as phenol oxidase and peroxidase, have been widely used for estimating the rates of phenolic degradation and SOM decomposition with N additions. Often, N enrichment decreases phenol oxidase [85, 95], while hydrolases are often activated. Sinsabaugh [45] reviewed that responses of phenol oxidase to N enrichment can differ by the types of ecosystem determined, as it decreases its activity in the forest and increases it in grassland or agricultural system. These contrasting results may originate from the initial lignocellulose contents in litter. In contrast, Bragazza et al. [96] have reported that N deposition can accelerate carbon release from peat bogs by activating phenol oxidase.

Global climate change models often predict increases in frequency and intensity of drought. Such changes can affect water availability in terrestrial ecosystems and water levels in wetlands. In wetlands, the effects of drought on nutrient cycling have drew much attention due to their close association with water. Most studies reported that drought increases the activity of phenol oxidase, implying stimulated decomposition [87, 97–99]. On the other hand, reduction in phenol oxidase activity was also found in peatland and heathland in response to simulated drought [4, 100]. Toberman et al. [100] suggested that initial water content in soils may be responsible for these contrasting responses and that a hyperbolic relation exists between water content and phenol oxidase.

7. Phenolics for Carbon Storage

Changes in the concentration, form, and decay rate of phenolics in response to climate can guide to better sequester

Table 3: Effects of environmental changes on phenolics and decomposition.

Environmental changes	Phenolics production	Phenolics degradation	Decomposition	References
CO_2	+/−			[83]
	+			[84]
	×			[101]
	+			[102]
	+			[103]
	+/×			[104]
	×	−		[105]
	+		−	[58]
	+			[82]
	+			[90]
	+			[81]
		+		[86]
		+	+	[87]
Warming	−			[90]
	×			[83]
		×		[106]
	+/−			[107]
	+			[89]
		−		[108]
N deposition	×	+/−		[105]
	+		−	[58]
			+/−	[45]
	×/−			[94]
	×/−			[92]
	−			[93]
Drought		+	+	[87]
	−			[109]
			−	[100]
		×/−	−	[100]
	+			[110]
			+	[98]
		+	+	[99]
CO_2 × Warming	×			[90]
	×/−			[83]
		−		[108]
CO_2 × N deposition		−		[111]
		−		[105]
		−		[58]
CO_2 × Drought		×		[87]
Warming × N deposition	−			[112]

+: stimulation, −: inhibition, and ×: no effect or interaction.

terrestrial C. Recently, Freeman et al. [7] have proposed that enhanced carbon storage in ecosystems, particularly in peatlands, is feasible by modifying phenolic contents which inhibit decomposition of organic matter by a mechanism called "enzymic latch" [113, 114]. They proposed that increases in phenolic content in peat ecosystems can be achieved either by increased expression of phenolic inhibitors from peatland plants or by enhancement of enzyme latch by physico-chemical modification. Furthermore, it is widely known that phenolic content can be enhanced by modifying pyrolysis conditions such as temperature, pyrolysis time, substrate, and oxygen supply for biochar preparation [115]. As such, we

propose that addition of biochar with high phenolics content represents a further approach to stabilize SOM in terrestrial ecosystems by inhibiting enzyme activities [116].

8. Future Studies Suggested

Studies of the ecological significance of phenolics have been conducted extensively since 1980, contributing significant understanding of their production, quantification, degradation, and effect on decomposition. As a secondary metabolite, phenolics have a range of structures and forms, with different reactivity. As such, application of appropriate methods for extraction and measurement must be applied according to the aims of each study. Assays to assess the activities of phenol oxidase and peroxidase have been developed to predict the degree and the direction of phenolic degradation.

However, there still remains controversy over how phenolics influence soil C cycling and how they are likely to respond to anticipated global environmental changes. For example, a larger supply of phenolics by global climate change may result in either faster or slower decomposition depending on the wider environmental conditions (Figure 3). Further, we conclude that the opposing trends of the effect of phenolics on SOM decomposition may be attributed to an insufficiently refined definition of the term "phenolics" or to the lack of information on redox mediators that control extracellular enzyme activities. We, therefore, propose that further studies are needed to understand fate of phenolics in response to simultaneous environmental changes with far higher resolution than in current practice. ^{13}C labeling may be an appropriate tool to elucidate phenolic turnover in soils. Additionally, molecular approaches aiming at specific genes for phenol degrading enzymes must be considered. Enhancing our knowledge about the role of phenolics following environmental change will facilitate a better understanding of nutrient dynamics in soils. Ultimately, such information can also be applied to techniques for carbon sequestration in terrestrial ecosystems by slowing down decomposition processes.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Vulnerability Assessment and Application of Bacterial Technology on Urban Rivers for Pollution Eradication

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To protect against the environmental pollution, the present research was undertaken to enumerate the Bacterial Technologies (BTs) on the restoration of polluted urban rivers, that is, Fenghu-Song Yang River (FSR) and Xuxi River (XXR). Experimental research accounted for the physiochemical parameters (pH; temperature; dissolved oxygen (DO); chemical oxygen demand (COD); total phosphorus (TP); total nitrogen (TN); and ammonia nitrogen (NH_3N)) before and after the BT operation. The results declared that the BT is efficient to restore the polluted rivers up to reliable condition. These results were analyzed by using multivariate statistical techniques (principal component analysis (PCA) and cluster analysis (CA)). These techniques interpreted the complex data sets and expressed the point source information about the water quality of these rivers at SA5, SA6, and SB3 under highly polluted regions. For better understanding, water quality index (WQI) was applied to compute the single numeric value. WQI results are evidence of the above results which prove the water quality of both rivers faced under outrageous condition (below 50 WQI scores) before the BT treatment, but, after the treatment, the rivers were restored from fair to good level (above 50 WQI scores) and overall output of these scores was quite similar to detect the point source of pollution. These results described an abrupt recovery of the urban rivers up to reliable condition for aquatic organism and clear effluents from the rivers.

1. Introduction

The urban rivers or streams have always been the recipient of sewage water from various sources that have different kinds of the domestic, agricultural, and industrial foreign particles [1]. The odor release from these water bodies stimulates the environmental pollution and the release of odor is often unavoidable due to its natural phenomena [2]. It is commonly known that raw municipal wastewater contains a great number of pathogenic and opportunistic microorganisms, as well as those antibiotic resistants including multidrug resistant, mainly of intestinal origin [3]. The urban river is an elemental source that collects the municipal wastewater with huge amount of sludge [4], the municipal sewage is the mixture of various organic matters, and the decomposition of these matters produces harmful gases that in fact deteriorate the environment and logically created the infection diseases [5]. The deterioration of water quality speedily contributes to water scarcity as a major concern. The Middle Eastern countries are facing the relative asperity of water quality according to various types of factors, including industrialization, nonrenewable water resources, population growth and density, institutional capacity, and economic situation. The constitutional expectation is that villagers have left their primitive residence, due to polluted potable water and food scarcity and skin and waterborne infectious diseases [6, 7]; therefore urban environment is becoming more alarming.

The fresh water resources are depleted because of massive agricultural activities, urbanization, and industrialization [8]. All over the world, the sustainable management of water is

a major concern for scientists, politicians, and social workers. In fact, worldwide, the natural processes and anthropogenic activities have been attributed to the surface water quality deterioration, including agricultural land use, hydrological features, sewage discharge, precipitation, and climate change [9–11]. Niemi et al. [12] stated that the human activities, that is, effluent discharge, eroded soil, and agricultural chemicals, are the major factors to deteriorate the surface and ground water. Pathogenic as harmful organisms are concerned in wastewater including bacteria, such as *Shigella* and *Salmonella* viruses and protozoa.

Therefore, to resolve the above issues would be a huge challenge for the planner and policy maker, due to treatment cost and the huge impact of unbridled population acceleration. Here we need a solution that efficiently resolves these critical pollution problems and could be used to rehabilitate the existence systems. Few decades ago, some conventional technologies have been developed and applied to treat the wastewater but these technologies failed due to huge maintenance cost and cannot control the huge effluent impact from various sources [13]. Thus, we need advanced and efficient system that could be helpful for today requirements. BT is an application of bioremediation that uses some beneficial bacterial metabolism to remove nutrients from the water bodies and regenerate the original condition [14]; BT operation cost is low with simple application procedure as compared to other conventional treatment systems [15]. Temperature is a major concern and is directly effective in the process of the degradation of the substances. In addition, beneficial bacteria are usually hired to vitiate pollutants or nutrients into simple, nontoxic substances and produce suitable effluents. BT has been successfully implemented to recover the lakes pollutant [13], restore polluted rivers, and assimilate effluent of wastewater treatment plant. It extends for the purification of polluted rivers and streams and also meeting the requirement on the standards of wastewater effluents without building massive structures as compared to the other conventional methods as needed in wetland construction [16].

The largest microbial community component based on bacteria in all biological wastewater treatment operations and quantity of these species is frequently encountered in the range of 106 bacteria/mL of wastewater [17]. These results are composed of complex and large data matrix of physicochemical parameters, which are usually interpreted with some meaningful techniques. The applications of multivariate statistical techniques, such as PCA, CA, factor analysis, and discriminant analysis, are helpful for interpretation of complex data frame into understandable form of ecology and water quality status of a county. These robust statistical techniques allow the recognition of the possible ways that can offer valuable tool for the rapid solution for pollution problem and water management as well as an influence on the water systems. Therefore, this paper briefly discusses practical implementation of BT on urban polluted rivers and argues with multivariate techniques and water quality index that BT is simple, affordable, and efficient for restoring polluted water bodies that directly or indirectly are a cause of environmental pollution.

2. Materials and Methods

2.1. Study Area and Sample Analysis

2.1.1. Site A. Fenghu (FH) and Song Yang (SY) Rivers are selected, FH River is placed on the tail of the SY River, and both of them are situated in Wenzhou (Rui'an) city (120°39.13′E and 27°46.49′N), Zhejiang Province, China. Most of the Wenzhou area is placed under the typhoon zone, and the FH River is taking water from Wenruitang and Liangmian River. The SY River is starting from cave bridge and falls directly into the FH River. The SY river length is about 280 m with the average breadth being 5–18 m and 1–3 m water depth, and FH river length is about 740 m with the average breadth being 6–15 m and 1 m water depth. For experiment monitoring and sample collection, the selected length from both rivers was divided into six points as 1 to 6 (Figure 1). The appearance of the river water color was blackish or greenish, and bubbles were blowing on the surface of water. These rivers are situated under the commercial and highly polluted area, and almost 2000 m^3 sewage water enters into them. Therefore, the average depth of sediments is 0.1 m and the rivers water quality was unsuitable for any purpose.

2.1.2. Site B. Earlier the name of Xuxi River (XXR) was Shaoxiangbanghe and it is situated in Wuxi city, Chang Nan District of China, as geographically (31°56.29′N and 120°28.14′E). Its upper stream starts from the Jing-Hang main canal and travels towards the ancient small canal. The selected river length is 1360 m with 4.5 m of upstream surface average width and about the average depth of 1.4 m. River is under north subtropical humid zone and is marked by muddy sediments. This zone is facing four distinct seasons with the phenomenon of climatic influence circulation.

In both cities of the selected sites, the cause of awful environment is the nonexistence of sanitation facilities for the community. This information was collected from the previous data that most of the quality parameters were retrogressed as compared to Class III of the Chinese National Standard (CNS) for Surface Water Quality (GB2828-2002) [18]. On the basis of current surface water quality standard, the rank of water quality in the source section was determined to be graded V (Table 1). Class V shows the worst (poor) and least water quality standard enlisted by the Chinese National Standard board. For any purposes, the river water is extremely unsuitable. Hence, this site selected for small urban rivers is in the worst category of Class V in China.

2.2. Bacterial Implementation. Bacterial Technology (BT) applies in a simple way; its procedure is held under three kinds of material as Bacterial Clusterization (BC), Nature Liquid, and biological filter media. BC is an important material that has a mixture of three types of ingredients as beneficial bacteria (Bacilli, *Bacteroides*, brown-rot spindle, and Lactobacillales, denitrifying with 6 : 4 : 3 : 4 : 3), mixing medium (catalyst process as glucose, sucrose, cellulose liquid, yeast cream, liquorice root, magnesium sulfate, dipotassium hydride, mannitol, tartaric acid (Na; K), folic acid, and ammonium nitrate), and water as in the ratio 4 : 3 : 3,

Table 1: Water quality parameters of both scenarios and Chinese National Standard.

Sampling time		Water temperature ˚C	pH	Monitoring project DO mg/L	COD mg/L	TP mg/L	TN mg/L	NH_3N mg/L
National Standard GB3838-2002		Class V index	6–9	2.00	15.00	0.0	2.00	2.00
FSR	14:40	16.1	7.5	2.5	10.90	0.96	14.80	10.60
	River water quality class		—	V	V	Inferior V	Inferior V	Inferior V
XXR	16:00	27.2	8.77	2.81	12.10	0.82	14.90	11.20
	River water quality class		—	V	V	Inferior V	Inferior V	Inferior V

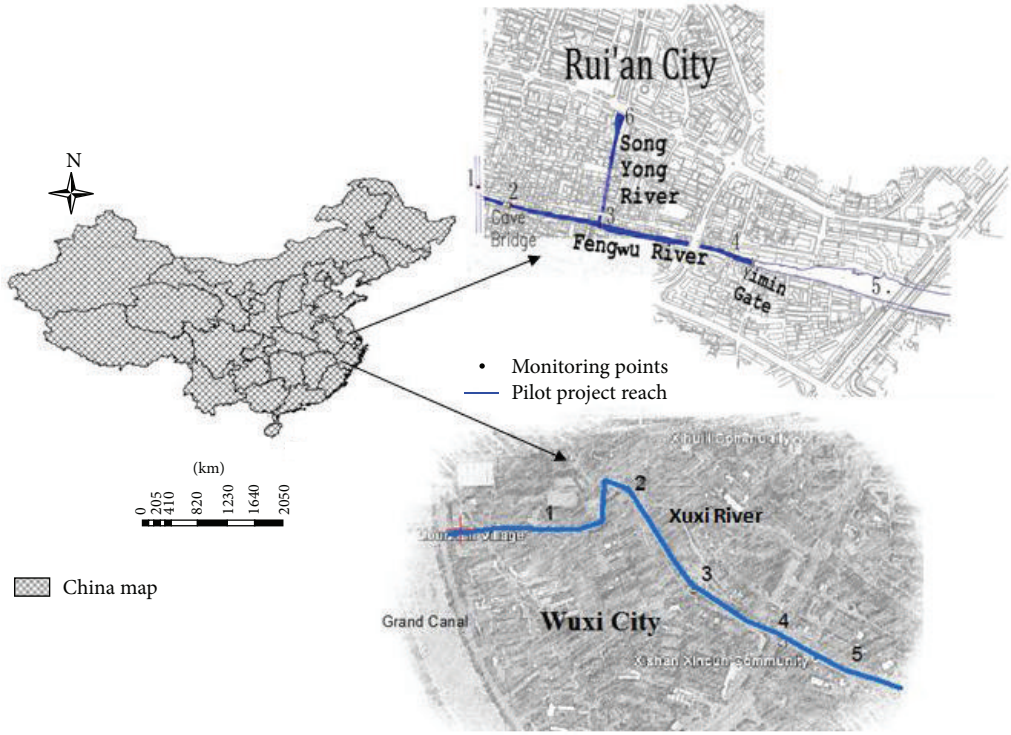

Figure 1: Study area and experiment site.

respectively [19]. The mixing ratio represents that it is harmless and has no adverse effects. Nature Liquid (NL) is the mixture of trace element, multiple enzymes, humic acid, amino acid, vitamins, and composition of each adequate substance on judgment. Biological filter media are used on a domestic level as the gap string filter media.

By implementation of BT on FSR, the 100 kg plastic drum was trained, mixed, and injected on the banks of the river as a proportion of the bacterial agent base (3 : 5 : 17) of aerobic and anaerobic cultivated bacteria for testing the suitable condition of the river water quality. Expanding culture of the diluted bacterial agent injected directly into the sediment at point "1" adopts a quincunx location method for monitoring positions. For successful results, a total of 12.9 tons of bacterial amount were employed on the FSR. 0.05% (bacterial agent) of bacteria were injected first time on November 18 to 20, 2011, and afterward 25, 27, and 30 as 0.025%, 0.02%, and 0.04%, respectively. Three times of the bacterial proportion were added on upstream station "1" as the specific bacterial quantity of 780 kg, 360 kg, and 560 kg, respectively. This site was monitored with the specific interval as 1, 2, 3, 6, 7, 8, 9, 10, 13, 14, 16, and 17 days during experiment and the random data collection at least 4 to 7 times in each month up to July 2012. The data was collected in almost 9 months.

By implementation of BT on XXR, a total of 11.1 tons of bacterial amount were employed on the selected points of the river (Figure 2). As the bacterial agent used effectively works under relatively constant and slow flow of velocity, an artificial weir was installed at the end of the river reach which is the small wood bridge to stop the sludge. It was technically built at about 50 cm height above the water surface level in order to extend the hydraulic retention time. This experiment was conducted at the end of May 2009. The XXR site data were collected with specific interval as shown in Figure 2 during the experiment and the data was randomly collected 4 to 7 times in the months after the operation of the BT.

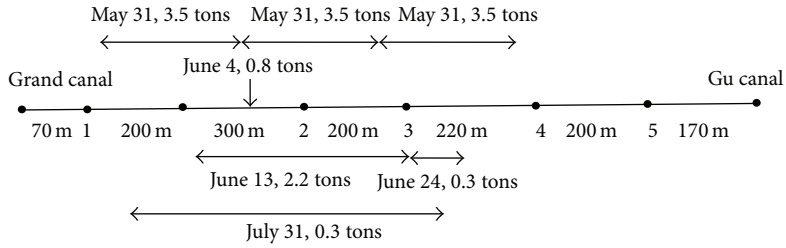

Figure 2: Schematic diagram of XXR and sampling points during BT operation.

2.3. Samples Collection and Pretreatment. The sampling network was arranged to cover the complete range along the inlet and outlet points of the rivers and determined the dominant point sources that have an impact on the water quality. Both of the sites are located under the area of population and industrialization, so the samples were collected from various depths (0.5 ft and >1 ft), at each monitoring station. The samples were collected at 8:30 AM to 4:30 PM during the period of the experiment and physiochemical parameters of temperature, color, pH, DO, COD, TN, TP, and NH_3-N were collected on the specific monitoring points of both rivers (Figure 1).

To determine the water quality, the samples were preserved in polyethylene bottles and stored in insulated ice cooler delivered to the laboratory on the same day. All the samples were saved at 4°C until the analysis and processing (ISO 5667-6, 1990; ISO 5667-2, 1991; ISO 5667-3, 1994). Then each parameter was tested in laboratory. The test methods were applied to determine the temperature (T), pH; and potassium dichromate oxidation for COD. Total phosphorus (TP), ammonia nitrogen (NH_3-N), and total nitrogen (TN) are commonly associated with wastewater. The growth of algae in large quantities can result from these nutrients. Also, depletion of oxygen concentration is caused by the growth of the algae matter. Therefore, the removal of these nutrients in wastewater becomes a critical part in effective water quality management for rivers.

2.4. Numerical Calculations for Data Treatment. All numerical calculation was analyzed by using Excel-2007 and xlstat-2014 software. Multivariate analysis of data set from the urban rivers' water quality was performed through PCA and agglomerative hierarchical clustering (AHC) technique [20]. The WQI approach was used for the better understanding of the water quality, before and after the bacterial restoration of the urban rivers.

2.4.1. Principal Component Analysis (PCA). PCA has the function of converting the original variables into new uncorrelated variables (axes), which are linear combinations of the original variables and lie along the directions of the maximum variance. PCA provides an objective approach to find this type of indices. So the variation in the data can be accounted for as concisely as possible [20]. PCA provides the information of the meaningful parameters which represents the whole aid data reduction and data set interpretation and summarize the correlation among constituents in the water with minimum loss of the original information. The first principal component loading displays most variance in the observed data, while each of the following components represents progressively less variance. PCA has been used in many regions for the understanding of the water quality [21, 22] and it provides the important information for the data interpretation.

Since the enormous variables of the data have been measured before and after the experiment related to the restoration of the urban rivers. Most of the data is raw and it is very hard to understand the environment conditions and historical changes to the data. Therefore, these techniques are useful, when large frame of data needs to be analyzed for the target area. In this research work, PCA was used to calculate the Pearson (n) correlation among components in the water samples of both urban rivers taken before and after the treatment.

2.4.2. Agglomerative Hierarchical Clustering Analysis (CA). The CA is an unsupervised technique which involves measuring the similarity or dissimilarity of the distance between the concerned objectives to be clustered. The resulting clusters of the objects should then exhibit high internal homogeneity and high external heterogeneity. Hierarchical clustering is the most common approach that is used for the instinctive similar relationship within the entire data set of the samples and illustrated by the dendrogram [20]. For the visual summary of the clustering process, dendrogram represents the agglomerative group drawing and its approximity, with a dramatic reduction in the dimensionality of the original data. The Euclidean method represents the analytical values usually used for the dissimilarity between two samples and distance [23]. In this research, the agglomerative hierarchical clustering technique was applied to the normalized data frame of both sites by means of Ward's method and spatial similarity detected by using squared Euclidean distance method, for the stations grouping under the monitoring of the experiment.

2.4.3. Water Quality Index. The surface water quality could be an intricate process undertaken to determine the different concerned parameters accomplished of large stresses capable of overall water quality of the river [24]. For pollution abatement, environmental management, and decision making, the water quality assessment plays a fundamental role. Therefore, to determine the water quality assessment, a water quality classification model should be developed firstly, and then we should apply the model to calculate the class of the water

Table 2: WQI criteria of ranking.

Rank	Excellent	Good	Fair	Marginal	Poor
WQI value	91–100	71–90	51–70	26–50	0–25

Table 3: Weighting ratio of water quality parameters for WQI.

Parameter	Temperature	pH	COD	TP	TN	NH_3N	DO
Wi	0.19	0.11	0.15	0.12	0.13	0.13	0.17

quality based on the index values. Multi-index assessment model was established to obtain an appropriate value of the water quality. Once the data are collected from the experiment, they further need to translate into simple and effective interpreted form. Water quality indices are the best tool to represent the data in a simple and understandable format [25].

Many sanitation and environmental foundations have formulated numerous water quality indices which are used all over the world to easily judge and better understand the overall water quality of the water body within a particular area promptly and efficiently [26]. Numerous indices are based on various techniques that summarized the result in single number: for example, the Canadian Council of Ministers of the Environment Water Quality Index (CCME-WQI), US National Sanitation Foundation Water Quality Index (NSF-WQI), Oregon Water Quality Index (OWQI), and British Columbia Water Quality Index (BCWQI) [25–28]. These indices are formulated with the specific weighting ratio with the comparison of all water quality parameters with the standard values and provide a single numeric value to the source water quality. In this research, the index was used to evaluate water quality of both urban rivers during the BT operation that was formatted by the US National Institute of Health for water quality ranking in the early 1970s. Using the following equation, the US National Institute of Health formed water quality grading:

$$\mathrm{WQI} = \sum_{i=1}^{n} w_i q_i, \tag{1}$$

where WQI stands for the water quality index, "w_i" is weighting ratio, "q_i" is the value acquired according to the relevant parameters, and "n" is the number of parameters. For calculating the values of the index, Table 2 demonstrates the ranking of the water quality. For the present research, the variable weighting ratio was calculated from Table 3 and classified each station based on specific rank as shown in Table 2.

3. Results

The selected urban rivers were situated under the appalling environment and the rivers' conditions were awful before the operation of BT and they were located in commercial area; also industries were located along the side of the rivers. The huge amount of sewage loading was directly entered into these rivers. There was no sanitation facility to control or dump the municipal sewage to fall into rivers, so that sewerage made a part of urban rivers without any preliminary treatment. Therefore, there is an urgent need to treat these urban rivers for pollution abatement and to rehabilitate the aquatic environment of the rivers.

BT has applied in three sessions (long summer to short winter) of the year on the selected rivers and water samples were collected during the treatment of the rivers. The range, mean, and standard values of each parameter are in Table 4, after determining the values, compared with the improved efficiency of nutrients before and after bacterial action which was 79%, 74%, 68%, 70%, and 65% of DO, COD, TP, TN, and NH_3-N, respectively. From the results, the DO was the most critical parameter for aquatic life of rivers that have maximum efficiency. To protect from the environmental pollution, DO also plays an important role in respiration of aquatic animals. Improved efficiency of TP, TN, and ammonia nitrogen also has favorable results. For more consideration, the color and algae also recovered as shown in Figures 3(a), 3(b), and 3(c).

3.1. Numerical Evaluation of the Experiment. The fundamental statistics of these restoration experiments contained 1485 total water samples (11 monitoring points × 4 data frequencies × 5 replications × 9 months) as before and after the BT operation and all data is summarized in Table 4. The data were collected during a period of 9 months and each station of the site was monitored with spatial as well as temporal variation. The PCA results are formulated chemical components based on Pearson (n) correlation matrix (Table 5). Three components of PCA analysis represent 96.72% and 96.47% of the variance in the sampling data of FSR and XXR, respectively (Figure 4). The eigenvectors classified the physiochemical parameters into three groups based on the PCA values: the first group contained temperature and pH, the second has DO and COD parameters, and the remaining TP, TN, and NH_3N are in the third group.

Agglomerative hierarchical clustering analysis was applied on both restoration sites of the water quality, to determine the spatial dissimilarity grouping for the monitoring stations along the rivers. The spatial variation results indicated three significant clusters for six sampling stations on FSR site, as stations 1–4 have two clusters such as (1-2) and (3-4) which shows low mutual dissimilarities as compared to the third cluster or (5-6) stations and on XXR, the five sampling stations into three statistical clusters as (2, 4, 5) have low mutual dissimilarity as compared to (1, 3) station (Figure 5). From these results, two main objectives solved for the assessment of the water quality. On XXR, the 3rd station was placed under the area of industrialization and there is a main horrific point of pollution along the river and the FSR; downstream stations were located under polluted and worse condition and all the domestic and industrial wastes were received there.

For a comparative analysis of before and after the restoration of the rivers, WQI was applied and evaluates the water quality assessment of the rivers. The results declared that the selected rivers' water quality was poor (Class V) in both sites, before the treatment process as explained in Table 2; their environment was polluted and may cause deterioration of the environment from these urban rivers. After the BT operation,

Table 4: Water quality data of FSR and XXR experiment data.

Parameters	GB2828-2002 (CNS)		Fenghu-Song Yang River data						Xuxi River data				
			SA1	SA2	SA3	SA4	SA5	SA6	SB1	SB2	SB3	SB4	SB5
pH	6.5–8.5	Mean	7.3	7.4	7.2	7.3	7.4	7.6	7.3	7.7	7.5	7.6	7.5
		Standard deviation	0.27	0.27	0.27	0.27	0.27	0.24	0.28	0.23	0.26	0.23	0.26
		Minimum	7	7	7	7	7	7	7	7	7	7	7
		Maximum	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.9	7.8	7.9	7.8
DO (mg/L)	2	Mean	1.69	1.69	1.83	2.14	2.46	2.39	0.65	0.77	0.76	0.63	1.06
		Standard deviation	1.08	0.9	0.9	1.16	1.38	1.39	0.21	0.38	0.58	0.36	0.57
		Minimum	0.4	0.4	0.5	0.5	0.5	0.5	0.4	0.2	0.1	0.2	0.1
		Maximum	3.3	2.7	2.8	3.3	4.1	4	1.2	1.6	1.7	1.6	1.8
COD (mg/L)	15	Mean	70.37	72.24	59.7	59.01	59.01	58.53	13.63	10.73	13.3	12.9	13.73
		Standard deviation	49.29	52.45	35.22	40.32	35.2	30.87	1.44	2.34	1.54	1.41	1.27
		Minimum	28.4	29.1	29	24.7	24	24	11	5.6	11.2	9.7	11.3
		Maximum	147.7	150.1	117.2	122	123	109	16.7	15.6	16.8	15.4	15.6
TP (mg/L)	0	Mean	0.91	0.84	0.83	0.76	0.84	0.78	1.23	0.72	1.14	1.13	1.27
		Standard deviation	0.57	0.5	0.47	0.47	0.42	0.37	0.26	0.13	0.16	0.17	0.15
		Minimum	0.14	0.14	0.13	0.15	0.4	0.4	0.7	0.5	0.87	0.83	1
		Maximum	1.86	1.57	1.44	1.47	1.45	1.3	1.66	1.02	1.44	1.46	1.49
TN (mg/L)	2	Mean	29.31	23.17	24.44	23.01	25.77	27.31	25.31	21.17	22.44	21.01	21.77
		Standard deviation	3.57	4.45	2.18	2.27	1.36	3.58	3.47	4.28	2.16	2.25	1.36
		Minimum	19.2	17.5	17.5	17.4	20.3	19.2	19.2	13.5	18.5	16.4	19.3
		Maximum	37.1	36.2	29.6	26.8	31.6	34.1	32.1	28.2	26.6	24.8	24
NH_3N (mg/L)	2	Mean	14.21	13.87	11.49	10.59	11.44	12.02	17.8	10.65	15.59	15.62	17.83
		Standard deviation	9.77	10.23	9.99	11.5	10.44	7.9	3.08	1.49	1.82	1.17	1.31
		Minimum	2.42	2.01	2.04	0.87	1.76	1.43	11.5	8.35	12	13.3	15.9
		Maximum	27.3	26.9	25	26.7	26.7	24	23.6	14.4	20.1	18.1	20.1
Temperature (°C)	15–20	Mean	16.5	16.43	16.43	16.46	16.7	15.5	25.9	27.2	26.4	26.2	26.2
		Standard deviation	0.32	0.36	0.29	0.34	0.35	6.23	3.33	3.49	3.38	3.39	3.45
		Minimum	16.2	16	15.9	16	16.4	15.2	25.8	26.6	25.8	25.7	25.8
		Maximum	17.1	16.9	16.8	16.8	17.4	16.7	26.5	28	27	27.1	27.5

Table 5: Eigenvalues and vector on correlation matrix of water quality variables in FSR and XXR.

Parameters	FSR components			XXR components		
	1	2	3	1	2	3
pH	**0.727**	−0.089	−0.076	**0.628**	−0.086	−0.186
Temperature	**0.463**	−0.345	0.035	**0.842**	−0.324	0.112
DO	0.143	**0.517**	−0.062	0.317	**0.928**	−0.023
COD	0.144	**0.837**	0.162	0.213	**0.532**	−0.864
TN	0.063	−0.093	**0.745**	0.171	−0.271	**0.868**
TP	0.157	−0.053	**0.827**	0.147	−0.138	**0.914**
NH_3N	0.241	−0.104	**0.771**	−0.139	0.034	**0.875**
Eigenvalue	4.23	2.42	1.13	5.09	1.15	0.51
Variability %	60.37	20.23	16.12	72.66	16.49	7.32
Cumulative	60.37	80.60	96.72	72.66	89.15	96.47

the water quality was restored up to reliable condition (Figure 6). To distinguish the classes of WQI scores, a red line was drawn for the better clarification and the scores below the line represent the poor class as red arrow shows the worse indication, but as to grow up the WQI scores, it declares the "fair to good" water quality as green arrow indicates that the BT operation is helpful to mitigate the pollution up to reliable condition.

4. Discussion

4.1. Urban Rivers Chemistry. All the physiochemical parameters were collected during the BT experiment. The minimum and maximum values of water samples are presented in Table 4, and the results from both sites are compared to the values of Chinese standards of surface water as recommended for the permissible limits of Classes I–V. Temperature is the main parameter to employ BT at any location; because the temperature increases, molecules move faster, enzymes speed up metabolism, and cells rapidly increase in size. But, above a certain value all of these activities are proceeding at such high rates, enzymes start to denature, and the total effect is

Figure 3: (a) Contrast diagram of Fenghu River. (b) Contrast diagram of Song Yong River. (c) Contrast diagram of Xuxi River.

detrimental. Cellular growth ceases. Therefore, in these concerned sites of urban rivers, the experiment was performed under the temperature range from 15°C to 30°C.

4.2. Site Station Grouping and Spatial Dissimilarity. To normalize the complex data frame of both sites, PCA was applied and differentiated by hierarchical cluster technique to evaluate the comparative composition pattern among the samples taken from the monitoring stations. In view of macroscopic consideration, all the physiochemical parameters have the same behavior as the high concentration of toxic nutrients in whole urban rivers, but the light variation could exist on the loading pollution with the temporal effects. For rapid assessment, CA technique is useful for each cluster of the whole network to evaluate the water quality. It is the evidence that both restoration sites are classified based on sampling station along the whole river and adequately divide these stations into specific optimal manner for BT evaluation.

The DO is the major concern under these restoration sites; the key parameters rehabilitate the aquatic environment and are also helpful to degrade the toxic metals. The negative loading of ammonium nitrogen was observed, whereas the strong positive loading on DO has up to 79% efficiency. Thus, the main advantage of the BT is that the bacteria have self-reproduction property that addresses the polluted and harmful particles from the urban bodies. The PCA trend obtained

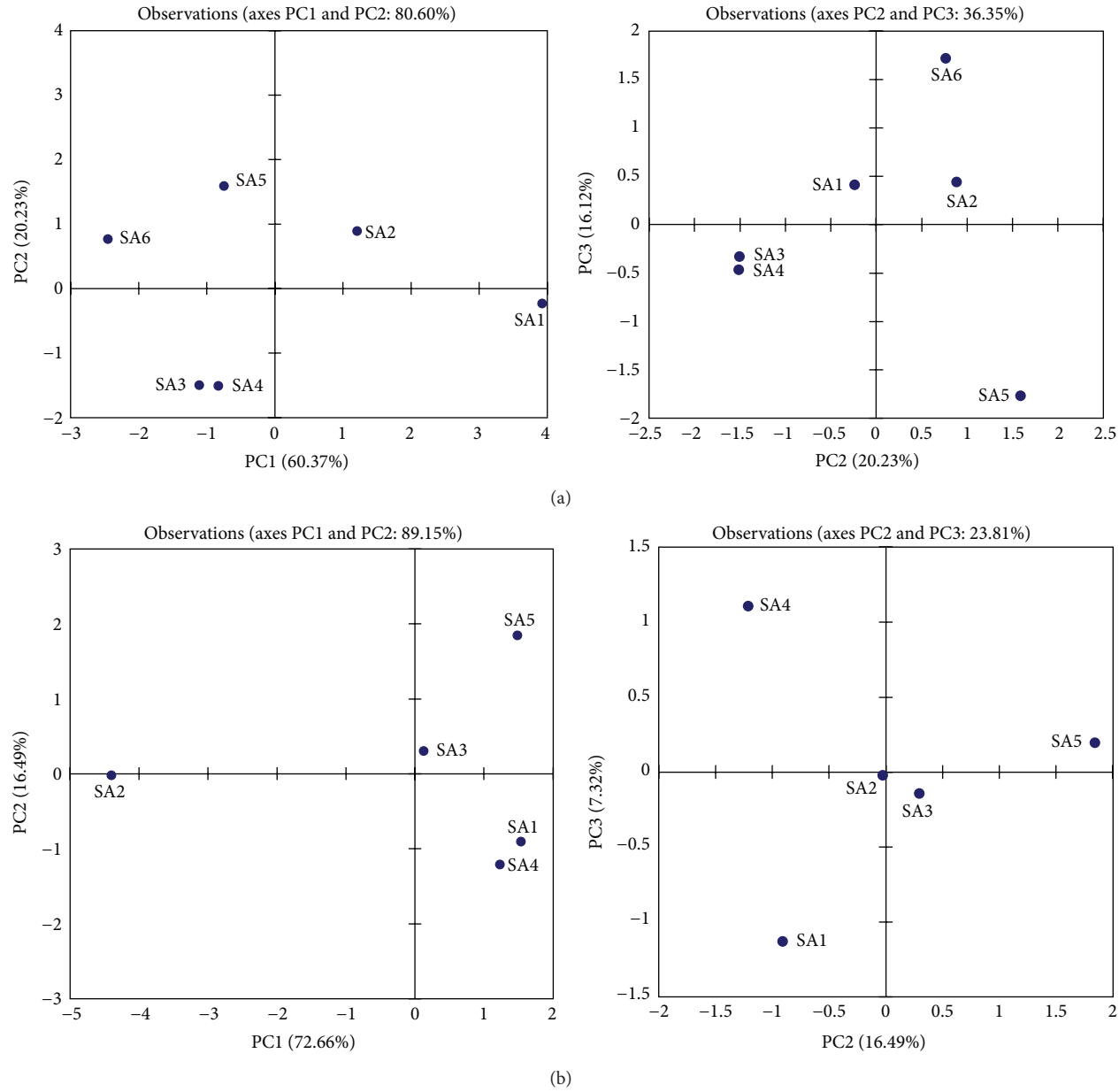

Figure 4: Scores of principal components: (a) FSR, (b) XXR.

was also helpful for the analysis of the raw data set. The second component shows the 20.23% in FSR and 16.49% in XXR of the strong loading of DO and COD in the total variation. The third component shows the 16.12% and 7.32% of the total variance in FSR and XXR, respectively, for the higher values of TP and TN are above the allowable limits of the Chinese standards values for the surface water quality.

The AHC results based on PCA scores clarify the abnormality in the dendrogram of both sampling sites with each monitoring station, which make the cluster groups with identical effluents variance from the various aspects of parameters. In FSR, the cluster group (5-6) was placed downstream under the number of nonpoint sources as agricultural, industrial, and domestic sewage. Besides the mutual dissimilarity among other clusters (1-2) and (3-4) have relatively low pollution status as compared to correspondence. Similarly, cluster group is verifying the pollution status in XXR upstream at SB1 and at the monitoring station SB3, the nonpoint sources take part in increasing the pollution due to anthropogenic activities and commercial area. The comparative results from both sites declared that monitoring stations (SA3, SA4), (SA1, SA2), (SA5, SA6) for FSR and (SB2, SB4), (SB5), and (SB1, SB3) for XXR have relatively low, medium, and high pollution regions, respectively. It implies that for rapid evaluation of the water quality only one station from each cluster may be used to describe the spatial evaluation of the overall network. For example, Tabata et al. research investigated the Ariake Sea, Japan [29]. They have interpreted 11 water quality parameters

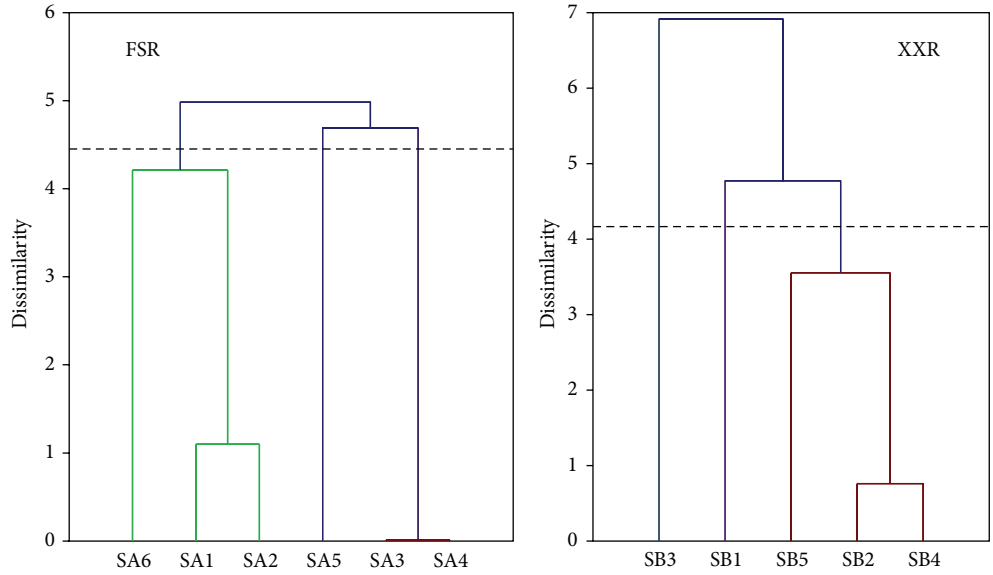

Figure 5: Dendrogram for agglomerative hierarchical clustering analysis.

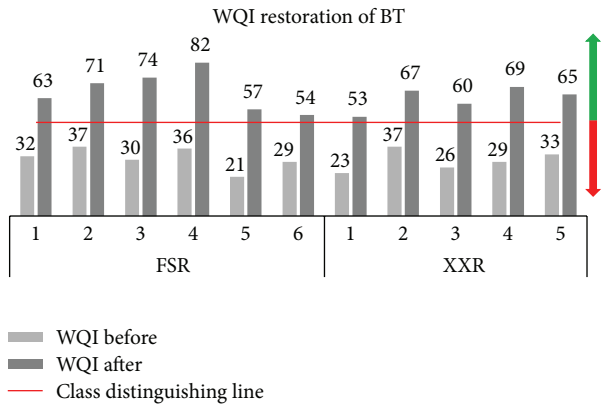

Figure 6: Water quality index on restoration of FSR and XXR.

by using PCA, to determine the organic pollution level and seasonal changes. Kazi et al. used multivariate techniques on Manchar Lake, Pakistan [20]. He interpreted PCA and CA with 36 water quality parameters to determine the deterioration cause of the lake water quality and anthropogenic activities impact on the Manchar Lake. Tabata et al. also evaluate the organic pollution and seasonal variation of the Ariake Sea through PCA and applied AHC to clarify the PCA scores. Therefore, it is evident that the AHC is a useful technique to provide helpful classification for the assessment of the whole region's water quality in an optimal pattern. Thus, the total number of monitoring stations and cost of the network could reduce without fixing of any significant outcome.

For better understanding and single numeric value, WQI was applied on both experimental sites which aim to reduce the complex data frame into optimal manner and enable interpretation of the monitoring data into a single numeric format. WQI results are revealed in Figure 6, which describes the pollution status of each monitoring station as before and after BT implementation. The WQI scores of FSR at 5 and 6 stations show that the values vary from 21 and 29 to 57 and 54 before and after the BT operation, respectively. Similarly, the WQI results of XXR site are expressed in Figure 6. It shows that the upper stream and middle of the river are under worse conditions and the WQI scores are varying from 23 and 26 to 53 and 60, respectively. It is evident from the above results that the river region is under an appalling environment with outrageous condition before the BT operation. The comparative results, before and after the BT operation, are quite similar to the above results as generated from AHC. Thus, the efficiency to restore the pollution from before to after BT could help to employ the amount of bacterial agent at any instant. Therefore, WQI was applied to evaluate the whole network of both urban rivers.

4.3. Cost Benefit Ratio Based on Conventional Technologies. Temperature is the major concern under the metabolism process of the bacteria, with higher temperature (20–35°C) during the summer from May to September and lower temperature (5–18°C) in the winter season from December to February. From the results, it is observed that the BT can perform the best outcome under 15–30°C temperature. The plus advantage of BT is that it does not need to demolish existing system. Therefore, new or existing systems could be easily integrated with the BT for continuous enhancement in the operation. BT has no effect on the natural environment because it does not involve the use of chemicals. Therefore, it is helpful for friendly ecology. All other issues, such as construction and high maintenance costs as other conventional systems have been operated that made a huge burden to sanitation foundation, government, and policy makers. Recent discussions on the urge for green technologies and climate change compel all the countries to use reliable technologies

that are reasonably sustainable. Sustainability stands to mean activities or development that meets the needs of the present without distrusting the needs of the future generation.

In view of these revolutionary calculations, the adoption of BT becomes the most convenient approach for developing countries as has been concluded [19]. The cost of treating the tons of wastewater is about 241≈321$ which WWTPs is being built, under construction and already built, the operation cost is between 0.12 and 0.22$ per ton [15]. According to this statement, the municipal sewerage operation system needs to spend above 16×10^7 $ on a single attempt. If the pipe network of municipal administration is built, the amount of total cost will exceed 32×10^7 $, and the operational cost is raised up to 4×10^7 $ annually. Therefore, the addition of bacteria to treat the sewerage wastewater, the one off investment cost is only 65$ and this method is simple, easy to operate, and affordable. For the long term, there is no need of maintenance and artificial dregs up to 10 years [15]. In addition, the existing sanitation systems are deteriorating due to many-imperfection care. So BT has the ability to restore these systems due to its self-purification property. Similarly the maintenance cost of the sewerage system is unfavorable due to economic collapse. So we can prefer BT due to its simplicity, affordability, ecofriendliness, and adoptability for any scale of system and existing program.

5. Conclusions

This research emphasized that the BT offers an innovative technique which provides an ingenious solution for the rehabilitation of the polluted urban streams. The results declared that both rivers were under outrageous condition of water quality that directly affects environment ecology, while after BT operation the worse condition of the urban rivers was rehabilitated. In this research, various multivariate techniques were applied to determine the spatial and temporal variations along the rivers. This study demonstrates interpretation of the problems of the complex data set through multivariate techniques because chemometric research enables us to discuss the similarities and dissimilarities along the observing stations among the variables that could not be clearly visible for assessment of the analytical data in a table. From overall results it is concluded that BT is efficient due to its simplicity and being economical, affordable, and reproducible on any scale of the operation. Hence, it is helpful to find reasonable, reliable, and efficient solution to the various future water pollution problems all over the world.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Evaluation of Natural Materials as Exogenous Carbon Sources for Biological Treatment of Low Carbon-to-Nitrogen Wastewater

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In the bacterial processes involved in the mitigation of nitrogen pollution, an adequately high carbon-to-nitrogen (C : N) ratio is key to sustain denitrification. We evaluated three natural materials (woodchips, barley grains, and peanut shells) as carbon sources for low C : N wastewater. The amount of organic matter released from these materials to aqueous media was evaluated, as well as their pollution swapping potential by measuring the release of total Kjeldahl nitrogen, N-NH_4^+, NO_2^-, and NO_3^-, and total phosphorous. Barley grains yielded the highest amount of organic matter, which also showed to be the most easily biodegradable. Woodchips and peanut shells released carbon rather steadily and so they would not require frequent replenishment from biological reactors. These materials produced eluates with lower concentrations of nutrients than the leachates from barley grains. However, as woodchips yielded lower amounts of suspended solids, they constitute an adequate exogenous source for the biological treatment of carbon-deficient effluents.

1. Introduction

Anthropogenic eutrophication is a major water pollution problem worldwide. Overenrichment of nutrients (nitrogen and phosphorus) increases the production of biomass in aquatic systems, thereby impairing the water quality and threatening the natural balance of these ecosystems. Agricultural runoff, livestock operations, aquaculture, industry (e.g., food processing facilities and pulp and paper mills), sewage treatment plants, and fossil fuel combustion are the major sources of nutrient pollution [1].

The mitigation of nitrogenous pollution mostly relies on biological treatments based on the well-known route ammonification-autotrophic nitrification-heterotrophic denitrification. In these processes, the carbon-to-nitrogen (C : N) ratio is a key design parameter. Although an approximate 10 : 1 ratio (measured as COD/TKN) is frequently recommended, some authors suggest values as high as 20 : 1 or 30 : 1 [2]. However, at excessively high C : N ratios heterotrophic bacteria can outcompete nitrifying microorganisms [3], whereas low C : N ratios limit denitrification and can cause the accumulation of NO_2^- in total nitrogen removal processes [4].

Many of the aforementioned pollution sources generate effluents with disproportionately high contents of nitrogen. For instance, wastewater from the optoelectronics industry is rich in organic nitrogen because ethanolamine and tetramethyl ammonium hydroxide are used in the manufacturing process [5]. Intensive aquaculture systems tend to generate effluents enriched in NH_4^+ [6, 7], as well as petrochemical, pharmaceutical, fertilizer, and food industries [8]. Stainless steel manufacturing processes generate wastewater with nitrate concentrations ranging from 500 to 1000 mg N-NO_3^-/L [9], while in agriculturally impacted groundwater a range of 1-2 mg N-NO_3^-/L is expected [10]. In the two last cases, hardly any organic matter is found.

Any unbalanced C : N wastewater requires the addition of exogenous carbon sources. A wide variety of compounds has been employed for this purpose, such as sugars, organic acids, alcohols, and oils [11], though methanol appears to be the most common [9]. Recently, solid materials have received more attention, and consequently cornstalks [11], wood by-products (e.g., sawdust and woodchips) [12], wheat straw [13], compost [14], and starch-based biodegradable polymers [15], among other substrates, have also been used as external carbon donors.

When selecting a carbon source, several aspects must be considered, such as its cost, denitrification rate, handling safety, and potential release of toxic compounds. In fact, the costs of the carbon donor and the sludge management are key, as they account for more than 50% of the overall wastewater treatment cost [9]. The performance of the treatment is often hindered by the export of excessive amounts of dissolved organic carbon from the source [16]; consequently, in order to guarantee a proper dosage of the material, the amount of organic matter leached must be assessed. Another important aspect of the carbon source to take into account is its pollution swapping potential, which is the increase in one pollutant concentration as a result of an action implemented to remove another pollutant [17]. Nevertheless, even if some natural materials have been extensively used as carbon donors, their potential of pollution swapping is rarely quantified.

We evaluated three economical, natural materials (woodchips, grains of feed barley, and peanut shells) as potential carbon sources for low carbon-to-nitrogen wastewater. In leaching tests, we measured the amount of organic matter released from these materials in aqueous media, as well as their cross pollution potential in terms of release of nitrogenous compounds (total Kjeldahl nitrogen, $N\text{-}NH_4^+$, NO_2^-, and NO_3^-) and total phosphorous (TP). In addition, the chemical characteristics of the raw materials and the leached organic matter were studied by elemental analysis and infrared spectroscopy.

2. Materials and Methods

2.1. Natural Materials. Woodchips, grains of feed barley (*Hordeum vulgare* L.), and peanut (*Arachis hypogaea*) shells were studied as potential carbon sources. Woodchips were obtained from untreated pine (*Pinus sylvestris*). According to screen analysis, 85.5% of the woodchips were retained over a 2 mm mesh sieve, while 10.4% passed through a 2 mm mesh sieve but were retained over a 1 mm mesh sieve. The remaining fraction (4.1%) passed through a 1 mm mesh sieve but was retained over a 0.6 mm mesh sieve. Barley grains (0.6 ± 0.05 cm) and peanut shells (3.2 ± 0.47 cm) were obtained, respectively, from Apan and Temascalapa, both in the State of Hidalgo, Mexico. All foreign matter (such as stones, dust, or stalks) and damaged kernels were removed from barley by hand.

Elemental composition and Fourier Transform Infrared (FT-IR) analyses were made on 1.5–3.0 mg samples of the natural materials previously ground to a homogeneous fine powder and dried at 105°C for 24 h using a 2400 Series II CHNS Elemental Analyzer and a Spectrum GX FT-IR spectrometer (both from Perkin-Elmer, Waltham, MA, USA), respectively. The IR spectra were obtained from KBr pellets (1 : 100 weight ratio of sample/KBr). The spectrometer was set to scan from 4000 to 400 cm^{-1}.

2.2. Batch Leaching Tests. Leaching tests were performed in batch mode at a solid-to-liquid ratio of 50 g/L. The materials were washed with distilled water and air-dried. Samples of the natural materials were added separately to 1 L of distilled water in glass flasks. The flasks were purged for 10 min with N_2, sealed, and then placed under agitation on an orbital shaker (Polyscience, USA) at 120 rpm and at room temperature for 50 days. Periodically, 100 mL samples of the supernatants were taken from each flask and maintained at 4°C until analysis.

After 50 days, the supernatants were completely withdrawn from the flasks, filtered, and separated in about 1 mL portions to be lyophilized by continuous freeze drying (Freeze Dry System, Freezone 4.5, Labconco, Kansas City, MO, USA) at –50°C and 133×10^{-3} mBar. Lyophilized leachates were analyzed for elemental composition and FT-IR spectroscopy as for raw natural materials (Section 2.1).

2.3. Chemical Analyses of Aqueous Leachates. Except where indicated otherwise, the analyses were made according to the *Standard Methods for the Examination of Water and Wastewater* [18]. Chemical oxygen demand (COD) was analyzed spectrophotometrically at 600 nm after digestion of the samples with $K_2Cr_2O_7/H_2SO_4$ (method 5520). Ultraviolet absorbance at 245 nm (UV_{254}) was measured in a 1 cm quartz cell (method 5910). Dissolved organic carbon (DOC) was measured with an infrared analyzer (Shimadzu, Japan). BOD_5 was determined in the OxiTop measurement system (WTW, Germany). For measuring TKN, the samples were digested at 400°C and distilled in a Gerhardt Vapodest 20 (Germany) unit to transform organic nitrogen into ammonium ions, which were further assessed by titration with 0.01 N HCl. $N\text{-}NH_4^+$ was assessed by the phenate method (4500-NH_3). The 4500-NO_2^- and 4500-P F methods were used for measuring $N\text{-}NO_2^-$ and TP, respectively. Finally, the content of $N\text{-}NO_3^-$ was determined by the phenoldisulfonic acid method [19].

3. Results and Discussion

3.1. Chemical Composition of the Raw Natural Materials. The elemental analysis of the raw materials showed that carbon and hydrogen contents were 50.56% and 6.36% for woodchips, 48.01% and 5.31% for barley grains, and 44.49% and 6.47% for peanut shells, respectively. Nitrogen was only detected in barley samples, where it accounted for 0.97%. This value is lower than those usually reported in the literature (e.g., 1.27–2.01%) [20]. In cereals, the N content is directly associated with the total protein content, which is about 8–13% in feed barley [21]. Although an N content of about 2.3% has been found in peanut shells [22], also mostly related to proteins, this element could not be detected in our samples.

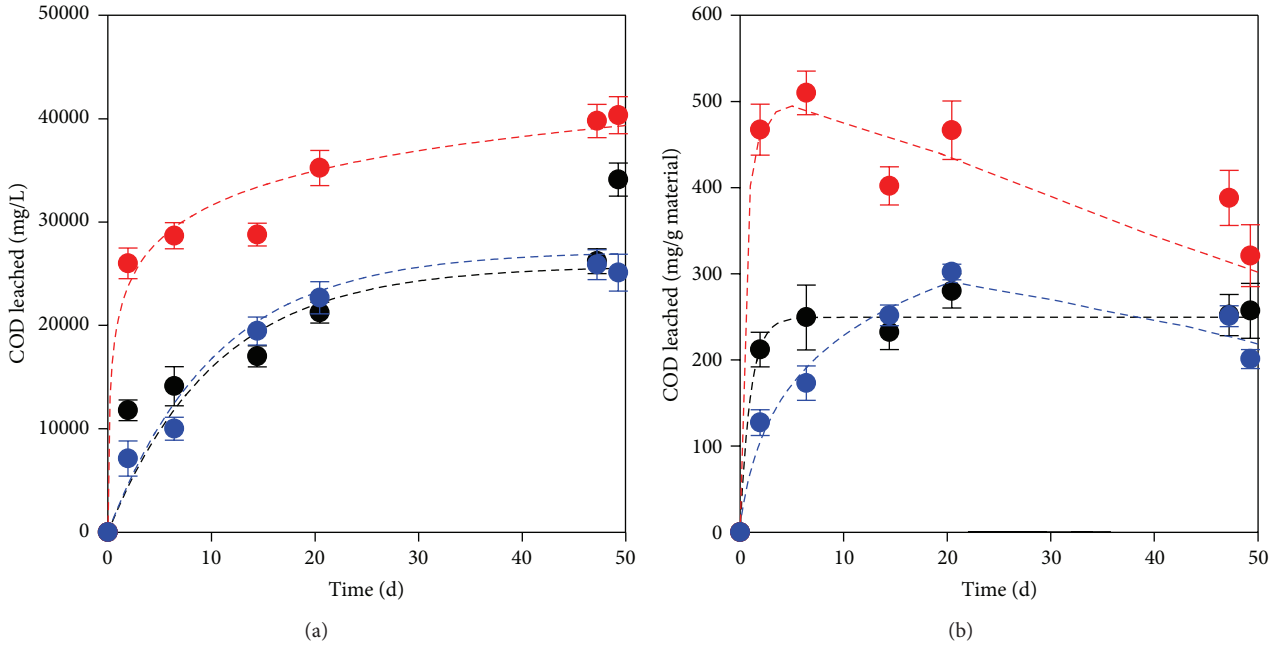

Figure 1: Leaching of organic matter measured as (a) concentration of COD and (b) mass of COD released per gram of natural material. Red bullet: barley grains, blue bullet: peanut shells, and black bullet: woodchips.

The FT-IR spectra of the three raw materials showed almost identical main signals. A broad band centered at 3400 cm^{-1} was detected, which is characteristic of the O–H stretching. A signal observed at 2920 cm^{-1} was attributed to C–H stretching, while the signal found at 1650–1640 cm^{-1} is characteristic of bending motions of absorbed water. A complex signal detected between 1470 and 1350 cm^{-1} was assigned to C–H bending and scissoring motions, and finally the strong signal at 1030–1020 cm^{-1} comes from C–O stretching. These signals are very well in agreement with the cellulosic nature of these natural materials. Indeed, cellulose accounts for about 34–48% of the mass of pinewood [23], 3.1–4.4% of barley grains [24], and about 35.2% of peanut shells [25].

3.2. Chemical Composition of the Lyophilized Leachates. During leaching tests, submerged plant material releases soluble compounds due to the breakdown of the vacuoles of plant cells by the physical action of water [26]. The analysis of both the elemental composition and the FT-IR spectra of the lyophilized 50-day leachates provided information about the compounds solubilized preferentially from the solid matrix.

Woodchips yielded the leachate with the highest carbon percentage (50.4%), followed by barley grains (26.7%) and peanut shells (21.7%). Concerning the nitrogen content, barley leachate presented the highest percentage (1.9%), which is consistent with the results of the elemental analysis carried out on the raw material. Peanut shells released an eluate with 1.5% of nitrogen, even though this element was not detected in the solid material by elemental analysis. In the leachate from woodchips, this nutrient was not found.

The FT-IR spectra of the woodchip and barley leachates showed no substantial change from their corresponding raw material. However, the FT-IR spectrum of the leachate of peanut shells showed a strong band at 1400 cm^{-1} that, along with a weak sharp band at 835 cm^{-1}, indicated the presence of nitrate. As this band was not detected in the raw material, it was hypothesized that the proteins of peanut shells were hydrolyzed and further ammonified to yield ammonium, which could be finally oxidized to nitrates by nitrifying bacteria. The leaching tests were conducted by preventing the entrance of oxygen into the flasks; yet, aerobic or microaerobic conditions could have been established, leading to nitrification. Although barley grains have higher protein content than peanut shells and the aforementioned bacterial processes could have occurred in barley leachate too, the FT-IR spectrum did not show the presence of nitrates. The results of both analyses suggest that barley grains and peanut shells could be inadequate carbon sources, because they might lead to a significant cross pollution due to the leaching of nitrogen compounds.

3.3. Leaching Kinetics of Organic Matter. Figure 1(a) shows the course of the concentration of organic compounds measured as COD in the eluates. In this figure, the typical biphasic curves displayed by natural materials in aqueous leaching tests are worth noting. In our assays, an appreciable concentration of organic matter was released from the three materials in the first 20 days; it accounted for 54, 89, and 86% of the COD content measured at the end of the leaching tests of woodchips, peanut shells, and barley grains, respectively. After 20 days, additional COD was still released but at a slower pace. This biphasic behavior has been also reported for other natural materials, such as wild sugar cane (*Saccharum spontaneous*) [26] and pine sawdust [27].

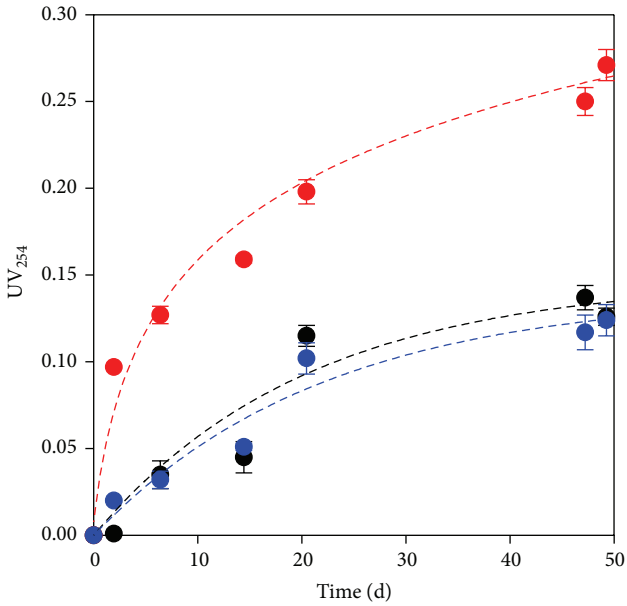

FIGURE 2: Organic matter (measured as UV_{254}) released from natural materials. Red bullet: barley grains, blue bullet: peanut shells, and black bullet: woodchips.

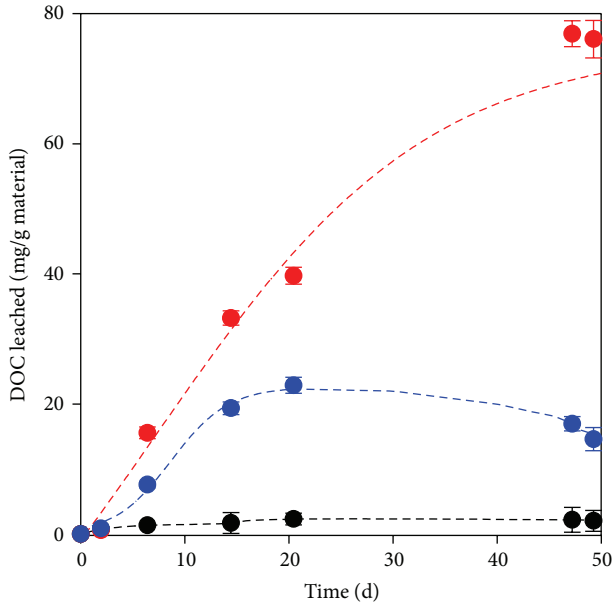

FIGURE 3: Organic matter (measured as DOC) released per gram of natural material. Red bullet: barley grains, blue bullet: peanut shells, and black bullet: woodchips.

Accordingly, the degradation of submerged organic materials seems to start with a leaching phase, which is followed by a hydrolysis phase characterized by the breakdown of the released macromolecules into simpler compounds.

Figure 1(b) shows the COD released per gram of each natural material. These data were obtained from mass balances taking into account the volume of medium extracted during samplings. For the leaching of COD from woodchips, the biphasic behavior was still observed, but the slow phase started earlier (after only two days). This is consistent with the results of Svensson et al. [27], who reported reaching an equilibrium in the leaching of organics from pine sawdust after 50 hours. The amount of COD released per gram of woodchips in the first two days of testing represented about 94% of the total COD released after 50 days. In the eluates of peanut shells and barley grains, after reaching a maximum release, a further consumption of COD was noticed. The depletion of the organic matter leached from barley started after the 6th day of testing; for the eluate of peanut shells, it started after 20 days. This implies that the leachate of barley grains is more biodegradable than that of peanut shells. Barley grains are mostly constituted by easily biodegradable macromolecules (carbohydrates: 60–80%; proteins: 13–16%; lipids: 1-2%) [28] and they have a low content of cellulose (a slowly biodegradable polymer); consequently, the aqueous leachate obtained from this material is expected to be also easily assimilable.

The release of organic matter was also assessed in terms of UV_{254} (Figure 2), because some organic compounds abundantly found in plants, such as lignin, tannins, and other phenolics, strongly absorb UV radiation [29]. Thus, UV_{254} was considered a surrogate for the organic matter released by the plant materials, which was supported by the strong correlation ($r^2 > 0.84$) found between our measurements of COD and UV_{254}. Other studies have also reported this correlation in wetland effluents [30]. However, as UV_{254} is related to aromatics, it is more closely associated with persistent organic matter (e.g., the humic fraction of natural organic matter) rather than total organic content.

As for COD, barley was the material that released the highest amount of aromatic organic matter (Figure 2). In fact, barley grains are good sources of phenolic compounds (between 450 and 1346 mg/g) [31] such as benzoic and cinnamic acids, flavonoids, tannins, coumarins, and resorcinols, which all can contribute to the UV_{254} value. In woodchips and peanut shells, the major source of phenolics is likely to be lignin, which represents 44.9% [32] and 36.5% [33] of these materials, respectively. In barley grains, lignin only constitutes 2.9% [34].

If the DOC is considered, barley was also the material that released the highest amount (Figure 3). Nonetheless, for this material, a linear DOC leaching was observed, rather than the aforementioned biphasic behavior. After 50 days of testing, the DOC accumulated was 2.0, 14.6, and 76.1 mg/g of woodchips, peanut shells, and barley grains, respectively. The accumulation of DOC released from woodchips was considerably smaller than the value (i.e., 45 mg/g) previously reported for pine wood chips after only two days of leaching [27], probably due to variations in the mean size of chips or in the solid/liquid ratios used in the leaching tests. The DOC released in our assays accounted for only 0.4, 0.8, and 17.1% of the initial carbon content measured by the elemental composition analysis of woodchips, peanut shells, and barley, respectively. For woodchips with several particle sizes (0.60, 1.18, and 4.75 mm), a prior study [35] had reported an organic carbon release of 1.1, 0.80, and 0.60% after 7 days,

respectively. Even though the leaching periods are different, this last value is consistent with our results, corresponding to woodchips with a mean size higher than 2.0 mm (85.5%). It has been advocated that woody materials are used more steadily than other natural sources because their carbon is not rapidly depleted [17]. In this way, they do not require frequent replenishment from biological reactors.

BOD_5 was measured only in the samples taken after 30 days of testing (data not shown). For these samples, the BOD_5/COD ratios of the leachates of woodchips, peanut shells, and barley grains were 0.15, 0.49, and 0.42, respectively. A BOD_5/COD ratio very similar (0.14) to the first value was reported for an old wood waste leachate [36]. According to these ratios, the organic matter leached from woodchips is less easily biodegradable than the organics leached from both peanut shells and barley. This is in agreement with the consumption of the COD signaled before for the eluates from peanut shells and barley grains (Figure 1(b)). Wood leachates contain a mixture of hemicellulose, lignin, tannins, and fatty acids, among other compounds [36]. The high molecular weight of some of these compounds could explain the low biodegradability of the leachates. In addition, it has been reported that wood leachates are toxic due to the presence of tannins, lignin, tropolones, terpenes, and lignans [36]; however, the performance of wood-like materials as carbon donors in biological treatments has been widely experienced [4, 11, 12].

3.4. Leaching Kinetics of Nutrients. Besides organic carbon, submerged plant materials are likely to release inorganic compounds. Figure 4 shows the amount of nitrogenous species released at the end of the tests (any concentration of nitrites was detected in the eluates).

After 50 days, barley grains yielded the highest amount of TKN among the materials tested, about 0.038 mg N/g. This can be attributed to the high protein content of this cereal, which is thereafter released into water. The concentrations of TKN released by woodchips (0.003 mg N/g) and peanut shells (0.005 mg N/g) are considerably lower than that produced by barley grains, because wood is composed mainly of organic molecules lacking nitrogen, such as lignin and polysaccharides. That is why nitrogen could be detected only in barley samples by the elemental analysis of the materials (Section 3.1). In fact, the poor N content of wood is one of the main causes of its environmental persistence, as nitrogen is the limiting growth factor for fungi required for exoenzyme production [37].

Even though the FT-IR analysis only evidenced nitrate in the leachate of peanut shells, we detected this ion in all the three eluates. As just stated, wood has a low content of N, and so the nitrates found in the leachate might come from some kind of foreign material. In the eluates of barley grains and peanut shells, nitrates were more likely to arise from the microbial oxidation of the organic nitrogen of these materials.

Peanut shells produced the least concentrations of total nitrogenous species, due to the low content of protein of this material (6-7%) [38]. Peanut shells are composed mainly of fiber (60–67%) [38] and their content of lignin (36.5%) [33] is

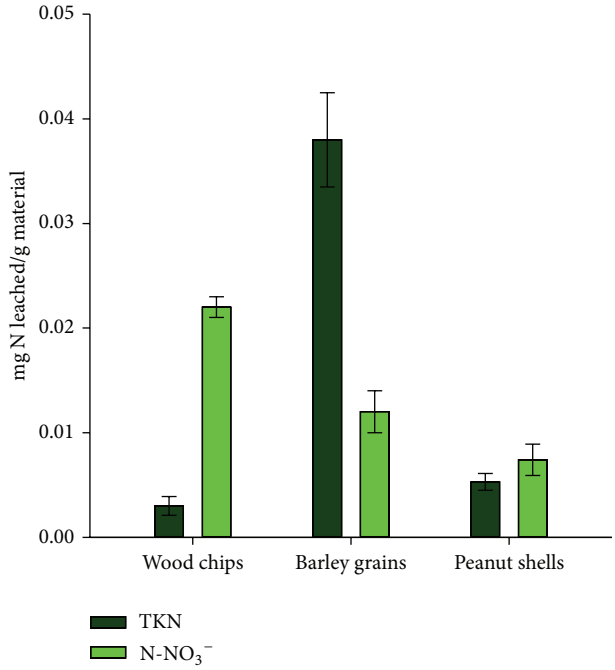

Figure 4: Nitrogenous species leached per gram of natural material after 50 days of testing.

even higher than that of most hardwoods and softwoods [39]. However, peanut shells disintegrate easily in aqueous media and produce high levels of suspended solids and turbidity (data not shown). Therefore, their use in biological reactors would require continuous replacement from the treatment system and would lead to a considerable pollution swapping.

When all the nitrogenous species (i.e., TKN plus nitrates) are considered, barley is the material that releases the highest amount of this nutrient to the liquid media (Figure 5). At the end of the leaching tests, the materials had released 0.05, 0.025, and 0.013 mg N per gram of barley grains, woodchips, and peanut shells, respectively. In the case of barley grains, for which an initial N content of 0.97% had been determined by elemental composition analysis, the release of N to the liquid media only corresponded to 0.7% of the initial input of this element.

The release of total phosphorus from the three materials is shown in Figure 6. Woodchips released barely detectable quantities of phosphorus throughout the experiment, whilst barley grains and peanut shells yielded about 0.30 and 0.13 mg TP per gram of material, respectively, at the end of the leaching tests. If the P contents of barley grains and peanut shells reported in the literature (0.37% [24] and 0.025% [22], resp.) are considered, it can be estimated that only 2.2% and 16%, respectively, of the initial input of this element were solubilized from the solid matrix after 50 days.

In this study, the release of organic matter and nutrients from submerged natural materials was studied quantitatively. Its main practical implication is the design of biological processes based on the use of these materials for treating low C : N wastewater with a stoichiometric approach. In this way, the release of carbon could be controlled to sustain the

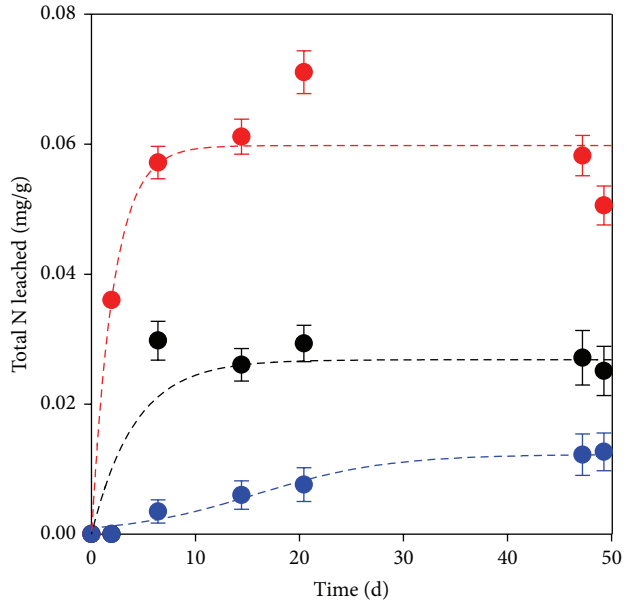

Figure 5: Total nitrogen released per gram of natural material. Red bullet: barley grains, blue bullet: peanut shells, and black bullet: woodchips.

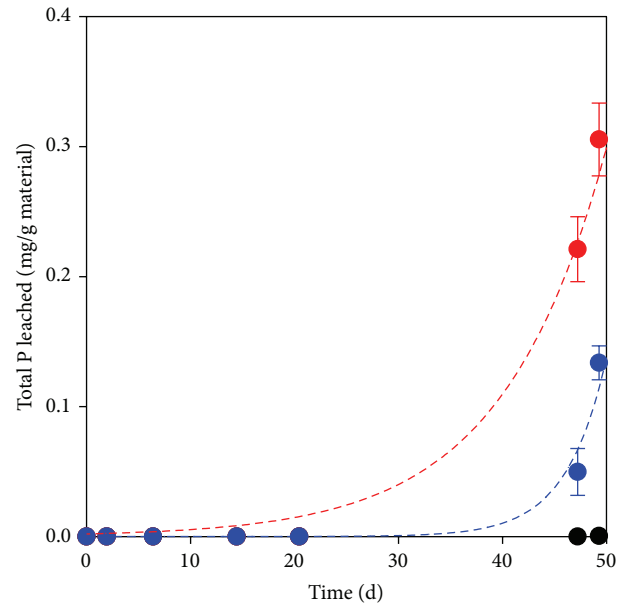

Figure 6: Total phosphorus (TP) released per gram of natural materials. Red bullet: barley grains, blue bullet: peanut shells, and black bullet: woodchips.

activity of all the bacterial groups involved in total nitrogen removal. For instance, the control of both the C : N ratio and the nitrate recycling ratio at optimal levels augmented the total nitrogen removal in biological aerated filters by enhancing the denitrifying activity [40]. In another study [41], the C : N ratio was controlled by a step-feeding strategy, which resulted in the increase of the total nitrogen removal in constructed wetlands. Similar results might be obtained in processes involving the natural materials studied here as external carbon sources for low C : N wastewater.

4. Conclusions

We evaluated three solid natural materials, woodchips, peanut shells, and barley grains, as potential carbon donors for the biological treatment of wastewater with an unfavorably low carbon-to-nitrogen ratio. On one hand, the analyses of the leachates indicated that woodchips and peanut shells are suitable carbon sources, as they release organic matter but lesser amounts of nutrients than barley grains. On the other hand, the organic matter released from woodchips is less easily biodegradable than that released by peanut shells and barley grains. The main drawback of peanut shells is that they disintegrate easily, thereby increasing the turbidity and the content of suspended solids of leachates. In conclusion, woody materials may be considered as adequate and economical carbon donors that minimize cross pollution in wastewater treatment.

Conflict of Interests

It is declared that the authors have neither any conflict of interests nor financial gain for this paper.

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Research Article

Decolorization of Distillery Spent Wash Using Biopolymer Synthesized by *Pseudomonas aeruginosa* Isolated from Tannery Effluent

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A bacterial strain was isolated from tannery effluent which can tolerate high concentrations of potassium dichromate up to 1000 ppm. The isolated microorganism was identified as *Pseudomonas aeruginosa* by performing biochemical tests and molecular characterization. In the presence of excess of carbohydrate source, which is a physiological stress, this strain produces Polyhydroxybutyrate (PHB). This intracellular polymer, which is synthesized, is primarily a product of carbon assimilation and is employed by microorganisms as an energy storage molecule to be metabolized when other common energy sources are limitedly available. Efforts were taken to check whether the PHB has any positive effect on spent wash decolorization. When a combination of PHB and the isolated bacterial culture was added to spent wash, a maximum color removal of 92.77% was found which was comparatively higher than the color removed when the spent wash was treated individually with the PHB and *Pseudomonas aeruginosa*. PHB behaved as a support material for the bacteria to bind to it and thus develops biofilm, which is one of the natural physiological growth forms of microorganisms. The bacterial growth in the biofilm and the polymer together acted in synergy, adsorbing and coagulating the pollutants in the form of color pigments.

1. Introduction

Molasses based distillery effluent contains intense quantities of recalcitrant pollutants in the form of dark colored organic pollutants. The intense color is due to the presence of a dark brown, acidic melanoidin pigment [1]. Melanoidin are a group of polymeric compounds which are a product of the Maillard reaction, a nonenzymatic reaction between sugars and amino compounds [2, 3]. The empirical formula of melanoidin is $C_{17\text{-}18}H_{26\text{-}27}O_{10}N$ [4]. These antioxidant and recalcitrant polymers cannot be easily degraded by conventional biological treatment methods, namely, anaerobic digestion (biomethanation), anaerobic lagoons, and activated sludge process [5, 6]. When the untreated effluent gets released into surface water resources, the dark coloration of melanoidin hinders the penetration of sunlight into the water, thereby decreasing the photosynthetic activity and eventually affecting the life of aquatic microbiome [7]. Moreover, the high concentrations of chemical oxygen demand (COD), biochemical oxygen demand (BOD), and biodegradable organic materials, namely, carbohydrate, lignin, hemicellulose, dextrins, organic acids, and obnoxious odor [8, 9], were also present in the spent wash effluent. Hence, disposing untreated spent wash effluent into the environment is unsafe to the ecosystem due to high pollution potential [10]. Physicochemical treatment methods involve adsorption, coagulation and flocculation, electrocoagulation, advanced oxidation, ozonation, membrane filtration, and evaporation. Adsorption and charge neutralization is one of the major physical-chemical treatment methods employed for removing pollutants and color.

Biopolymer belongs to the polyesters class which is produced by microorganisms. The types of aliphatic polyesters are Polyhydroxyalkanoates (PHA), Polycaprolactone (PCL), and Polylactic acid (PLA). Polyhydroxyalkanoates (PHA) are hydroxyacid polyesters that are synthesized and accumulated as intracellular granules by a wide variety of bacteria [11]. Of the big family of PHAs, Polyhydroxybutyrate (PHB) is the most widespread and well characterized [11]. PHB has aroused much interest in industry and research as a biocompatible, biodegradable, thermoplastic, and piezoelectric polymer with potential applications in medical, agricultural, and marine fields. Generally, the production of PHB is enhanced when a suitable carbon source is available in excess, but the cellular growth is limited by another nutrient such as nitrogen or phosphorus [11, 12]. Some bacteria can accumulate up to 60–80% of their weight as PHB [13]. Of the big family of PHA, a homopolymer of 3-hydroxybutyrate, poly-3-hydroxybutyrate (PHB), is the most widespread and the best characterized. The polyester PHB is synthesized and accumulated as intracellular granules by a wide variety of bacteria. It is generally accepted that microorganisms isolated from a natural environment are poor in nutrient sources and these microorganisms exhibit higher survival abilities than those living in the alimentary tract of higher organisms. It is well recognized that this lipid inclusion is accumulated by bacteria as they enter the stationary phase of growth to be used later as an internal reserve of carbon and energy. Among the factors restricting the economy of PHB production is the cost of the carbon source. Hence, there arises a lookout for a suitable and inexpensive carbon source for bulk production of microbial PHB.

As PHB is produced from the microorganisms, they are well supported in the development of bacterial biofilm which is one of the natural physiological growth forms for microorganisms over these polymer structures. By using this biopolymer as support material, the biofilm can be enhanced to develop well and it is interesting to use a microbial film immobilized on a micro-carrier surface for the production of a wide variety of biochemicals that can be utilized for other different purposes. One of the natural physiological growth forms for a microorganism is a biofilm, in which the microbial community is attached to a solid surface. From the biotechnological point of view, it is interesting to use a microbial film immobilized on a surface as a support material for the production of a wide variety of biochemicals that can be utilized for different purposes [14].

The objective of this study focuses on isolation, identification, and characterization of chromium tolerant bacterial strain from tannery effluent. Lab scale production of PHB using the isolated bacterial strain uses spent wash as the sole carbon source. Degradation of organic pollutants in terms of spent wash color uses PHB produced using the isolated bacterial strain.

2. Materials and Methods

2.1. Collection of Tannery Effluent Sample. The tannery effluent sample was collected from Pallavaram Tanners Industrial Effluent Treatment Co. (PTIETC) located near Chromepet, Chennai, India. This facility treats 3000 m^3/day of tannery effluent from the leather processing industrial cluster located nearby. Sample from the activated sludge tank were aseptically collected in sterilized glass bottles and transported to the laboratory and stored in the refrigerator at 4°C.

2.2. Collection of Distillery Effluent Sample. The distillery effluent sample was collected from Trichy Distilleries and Chemicals Limited (TDCL), located near the city of Tiruchirappalli, India. The collected effluent was immediately brought to the laboratory and stored in the refrigerator at 4°C [15, 16] until further use in order to avoid any deterioration in the physicochemical property of the spent wash.

2.3. Isolation of Metal Tolerant Bacterial Strain from Tannery Effluent. The metal tolerant bacterial strain was isolated by selection pressure method [17]. Chromium in the form of potassium dichromate ($K_2Cr_2O_7$) was added in varying concentrations of 10–2000 ppm to sterile nutrient agar (pH 7.0). The plates were loaded with 500 μL of raw effluent and the media was cast by pour-plate method. The colonies developed were counted after 3–7 days of incubation at 28°C. It is possible that some of the organisms die off due to pour-plate method. Consequently, the numbers of Cr (VI) resistant bacterial colonies able to grow were viewed on relative or comparative basis. The increasing concentration of chromium in the growth medium was given as a stress to resist the growth of the microorganisms. The strain capable of growing at maximum concentration was isolated. The isolated bacterial strain was identified with reference to *Bergey's Manual of Determinative Bacteriology* [18].

2.4. Molecular Characterization. The 24-hour fresh *Pseudomonas* sp. culture was taken for genomic DNA extraction based on isolation protocol described by Pitcher et al. [19]. The extracted DNA sample was run on 1% agarose gel with 1 k standard marker acquired from Bangalore Genei Private Limited, India. The universal primers were used to amplify the 16S rRNA gene region. The PCR amplification of 20 μL reaction mixture containing 1 μL of the template, primers: 2 μL of forward primer, U3 (5′AGTGCCAGCAGCCGCGGTAA3′), 2 μL of reverse primer, U4 (5′AGGCCCGGGAACGTATTCAC3′) [20], 12 μL of assay buffer, 1 μL of *Taq* DNA polymerase, and 2 μL of dNTP mix. The amplification was carried out in Thermal Cycler for 35 cycles using the following reaction conditions, *namely,* initial denaturation of DNA at 95°C for 5 minutes, denaturation of DNA at 95°C for 30 seconds, primer annealing at 45°C for 90 seconds, and primer extension at 72°C for 1 minute. The amplified PCR product was mixed with 2 μL of gel loading buffer and 1% agarose gel was cast. The samples were loaded along with 2 μL of 1 kb DNA ladder as a molecular marker. The gel was run and examined on a UV transilluminator to visualize the bands. PCR products were purified by using EZ-10 spin column PCR purification kit and it was sequenced.

2.5. Production of PHB. Vincent [21] proposed the composition of minerals and nutrients to be used in yeast extract mannitol (YEM) broth for the production of PHB.

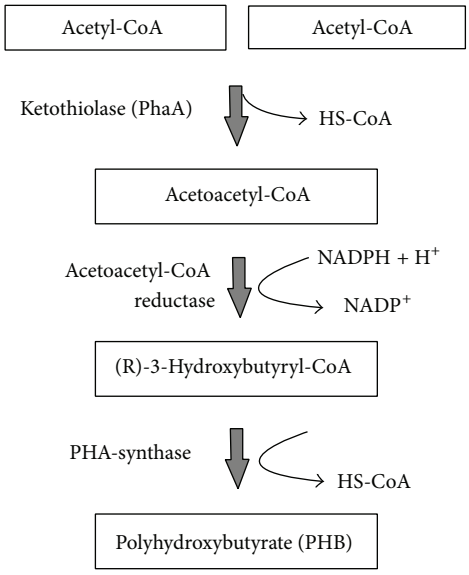

Figure 1: Biosynthetic pathway of Polyhydroxybutyrate (PHB).

The isolated and identified bacterial strain from tannery effluent was used for the production of PHB. Yeast extract mannitol (YEM) broth (g/L) consists of following ingredients: mannitol, 10 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.1 g; tryptone, 2.5 g; peptone, 2.5 g; yeast extract, 2.5 g. The pH of the medium was adjusted to 7.0 with dilute HCl. The batch production of PHB was carried out in 250 mL Erlenmeyer flasks containing 100 mL of culture medium. The temperature was maintained at 30°C and the culture was agitated at 110 rpm. The production medium was inoculated with a loopful of isolated bacterial culture. The biosynthetic pathway of PHB is shown in Figure 1.

2.6. Harvesting and Assay of PHB. The isolated, metal tolerant bacterial strain was cultured in YEM broth at 30°C for 48 hours in an incubator shaker. Cultures at stationary phase of growth were centrifuged at 6000 ×g for 45 min. The cell-free supernatant was discarded. The cell pellets were suspended in 5 mL of deionised water and homogenized for 2 min in a sonicator bath. To 2 mL of the cell suspension, 2 mL of 2 N HCl was added and boiled for 120 min in a water bath. The tubes were centrifuged at 6000 ×g for 20 min. To obtain precipitate, 5 mL of chloroform was added. The test tubes containing the suspension were left overnight at 28°C on a shaker at 150 rpm. The contents of the test tubes were centrifuged at 6000 ×g for 20 minutes and 0.1 mL of chloroform extract was dried at 50°C. About 5 mL of concentrated sulfuric acid was added and heated at 100°C in water bath for 20 min. After cooling to room temperature, the amount of PHB was determined using UV-Vis spectrophotometer at a corresponding wavelength of 235 nm. The schematic stepwise procedure for PHB harvesting is shown in Figure 2.

2.7. Determination of Dry Cell Weight. The total dry weight (total biomass) was determined by harvesting, washing, drying to constant volume, and weighing. The non-PHB dry

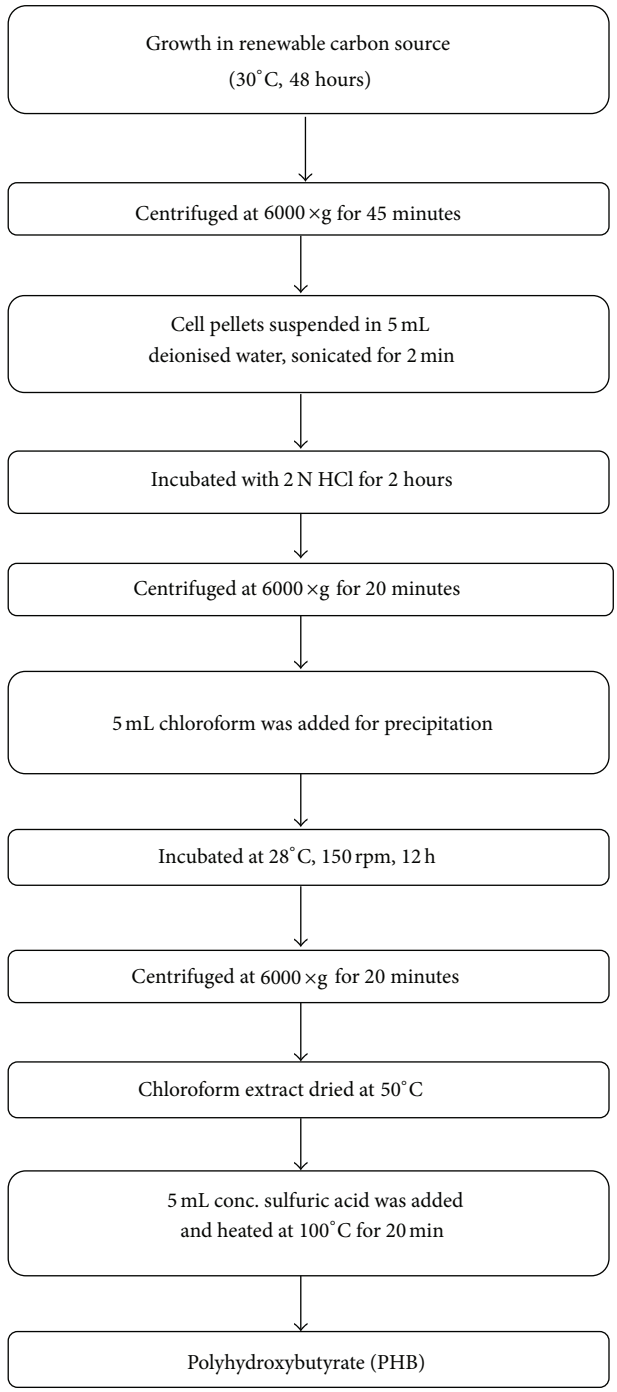

Figure 2: Schematic flow diagram representing harvesting and purification of PHB.

weight (non-PHB biomass) was calculated from the total dry weight and the PHB content using the following equation:

$$\text{Non-PHB dry weight} = \text{total dry weight} \times \frac{(100 - \%\text{PHB})}{100}. \quad (1)$$

2.8. Effect of Different Carbon Sources on PHB Production. The usage of mannitol in YEM medium broth was replaced by

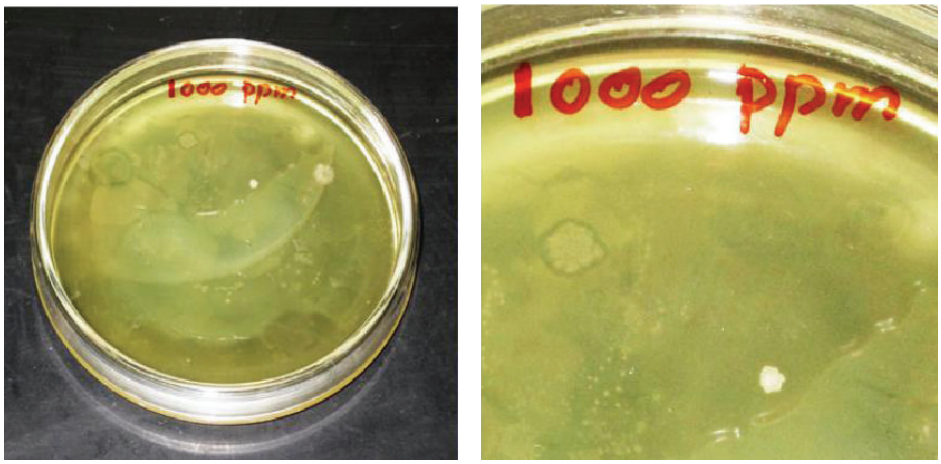

Figure 3: Microbial colony developed at 1000 ppm of $K_2Cr_2O_7$ dosage concentration.

other carbon sources such as glucose, fructose, dextrose, and sucrose in the growth medium. Peptone and tryptone were kept as constant nitrogen sources. Based on the well-known fact that molasses is rich in carbon source and inexpensive, trials were performed in which the expensive carbon source has been replaced by inexpensive molasses. The PHB yield for different carbon sources and molasses was determined.

2.9. Spent Wash Decolorization Studies. The batch color removal experiments were performed in Erlenmeyer flasks (250 mL volume) containing 100 mL of raw spent wash. An appropriate dosage of as-synthesized PHB and 48-hour-old bacterial culture was added as listed below:

(1) 2 mL of *Pseudomonas aeruginosa* culture.

(2) 2 mL of PHB synthesized using the isolated strain.

(3) 2 mL (1 : 1 ratio) of PHB and *Pseudomonas aeruginosa* culture.

The batch vessels were shifted to an incubator shaker and the flasks were mildly shaken at 50 rpm. Color reduction was monitored for 120 h. Aliquots of samples were withdrawn and centrifuged at 10000 ×g for 10 min to remove the suspended particles. Color removal was measured at a characteristic wavelength of 475 nm using UV-Visible spectrophotometer (Spectroquant, Pharo 300, Merck).

The color removal efficiency was calculated by

$$\text{Color removed (\%)} = \frac{C_0 - C_t}{C_0} \times 100, \quad (2)$$

where C_0 and C_t are the initial absorbance and absorbance at time t for spent wash effluent at a characteristic wavelength of 475 nm [22, 23].

3. Results and Discussion

3.1. Isolation of Metal Tolerant Strain. By selection pressure method, the most tolerant bacterial strain was isolated from tannery effluent. This strain was found to tolerate a maximum concentration of 1000 ppm (1000 μg/mL) of $K_2Cr_2O_7$, when cultured in nutrient agar media containing $K_2Cr_2O_7$ as shown in Figure 3. This method is to enhance the selection pressure, thereby reducing the number of surviving species, and only to obtain the organism that can withstand such high concentration (1000 ppm) of $K_2Cr_2O_7$. At lower concentrations, numerous well developed colonies were visualized. But, at 1000 ppm of concentration, only very few numbers of colonies were formed. These highly tolerant colonies were subcultured and preserved for identification, molecular characterization of the strain, and production of secondary metabolite.

3.2. Identification of Isolated Bacterial Strain. The microorganism isolated by the selection pressure method was identified by performing morphological, microbial, and biochemical tests and the results were compared with *Bergey's Manual of Determinative Bacteriology*. The colonies formed by the isolated strain were irregular circular in shape, with flat colony elevation, with uneven or rough colony margin, and dull white to mild beige in color. The microorganisms were identified to be Gram-negative motile rods as shown in Figure 4. The strain isolated tested positive in catalase test, due to the rapid evolution of gas bubbles, when a drop of H_2O_2 was placed on the bacterial colony, showing that there was an evolution of oxygen and the strain is aerobic. When subjected to oxidase test, the result was positive. This is due to the formation of dark blue, purple color which indicates the presence of cytochrome c oxidase. Indole test gave a negative result as there was no formation of the cherry red colored ring when Kovac's reagent was added to the incubated culture. Phenol red test result was negative as the isolated strain cannot ferment any of the sugars like glucose, sucrose, or lactose. So there was neither a change in color nor formation and collection of gas inside the inverted Durham's tubes. The result was methyl red negative upon performing methyl red test as there was no red color formation upon addition of methyl red indicator which denotes the fact that the pH

remains above 6.0. Formation of colorless colonies was seen when they were grown on EMB and MacConkey agar plates. This is due to the reason that the organism cannot ferment lactose sugars. The biochemical tests and the corresponding results are tabulated in Table 1.

3.3. Molecular Characterization. The PCR sequenced product was identified using Bioinformatics tool, BLAST, and *Pseudomonas aeruginosa* gene for 16S rRNA, partial sequence with 98% query coverage and 99% identity with expected value of zero was found. This confirmed that the organism is *Pseudomonas aeruginosa*. The sequence is given as follows and the BLAST results are shown in Figure 5:

GCAGGCCTAACACATGCAAGTCGAGCGGAT-
GAAGGGAGCTTGCTCCTGGATTCAGCGGCGGAC-
GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGT-
GGGGGATAACGTCCGGAAACGGGCGCTAATACC-
GCATACGTCCTGAGGGAGAAAGTGGGGGATCTT-
CGGACCTCACGCTATCAGATGAGCCTAGGTCGG-
ATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAG-
GCGACGATCCGTAACTGGTCTGAGAGGATGATC-
AGTCACACTGGAACTGAGACACGGTCCAGACTC-
CTACGGGAGGCAGCAGTGGGGAATATTGGACAA-
TGGGCGAAAGCCTGATCCAGCCATGCCGCGTGT-
GTGAAGAAGGTCTTCGGATTGTAAAGCACTTTA-
AGTTGGGAGGAAGGGCAGTAAGTTAATACCTTG-
CTGTTTTGACGTTACCAACAGAATAAGCACCGG-
CTAACTTCGTGCCAGCAGCCGCGGTAATACGAA-
GGGTGCAAGCGTTAATCGGAATTACTGGGCGTA-
AAGCGCGCGTAGGTGGTTCAGCAAGTTGGATGT-
GAAATCCCCGGGCTCAACCTGGGAACTGCATCC-
AAAACTACTGAGCTAGAGTACGGTAGAGGGTGG-
TGGAATTTCCTGTGTAGCGGTGAAATGCGTAGA-
TATAGGAAGGAACACCAGTGGCGAAGGCGACCA-
CCTGGACTGATACTGACACTGAGGTGCGAAAGC-
GTGGGGAGCAAACAGGATTAGATACCCTGGTAG-
TCCACGCCGTAAACGATGTCGACTAGCCGTTGG-
GATCCTTGAGATCTTAGTGGCGCAGCTAACGCG-
ATAAGTCGACCGCCTGGGGAGTACGGCCGCAAG-
GTTAAAACTCAAATGAATTGACGGGGGCCCGCA-
CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCA-
ACGCGAAGAACCTTACCTGGCCTTGACATGCTG-
AGAACTTTCCAGAGATGGATTGGTGCCTTCGGG-
AACTCAGACACAGGTGCTGCATGGCTGTCGTCA-
GCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGT-
AACGAGCGCAACCCTTGTCCTTAGTTACCAGCA-
CCTCGGGTGGGCACTCTAAGGAGACTGCCGGTG-
ACAAACCGGAGGAAGGTGGGGATGACGTCAAGT-
CATCATGGCCCTTACGGCCAGGGCTACACACGT-
GCTACAATGGTCGGTACAAAGGGTTGCCAAGCC-
GCGAGGTGGAGCTAATCCCATAAAACCGATCGT-
AGTCCGGATCGCAGTCTGCAACTCGACTGCGTG-
AAGTCGGAATCGCTAGTAATCGTGAATCAGAAT-
GTCACGGTGAATACGTTCCCGGGCCTTGTACAC-
ACCGCCCGTCACACCATGGGAGTGGGTTGCTCC-
AGAAGTAGCTAGTCTAACCGCAAGGGGGACGGT-
TACCACGGAGTGATTCATGACTGGGGTGAAGTC-
GTAACAAGGTA

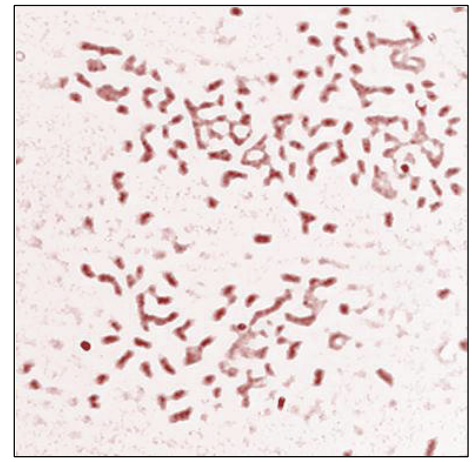

Figure 4: Gram's staining showing Gram-negative rods of isolated *Pseudomonas aeruginosa*.

3.4. Production of Polyhydroxybutyrate (PHB) by Using the Isolated Bacterial Strain. Pseudomonas aeruginosa, Gram-negative motile rod shaped bacteria, were able to synthesize Polyhydroxybutyrate (PHB) as an intracellular secondary metabolite which is a resultant product due to the physiological stress occurring due to the availability of excess amount of carbon source and limited availability of other minerals especially phosphate or nitrogen. It has been suggested that ammonia limited cultures of *Pseudomonas aeruginosa* were unable to regulate fully the rate at which they take up glucose, particularly when growing at the low availability of minerals. As a result, they form copious amounts of exopolysaccharide, both to overcome the potentially deleterious osmotic effects of accumulating surplus intracellular metabolites and to consume some of the surplus ATP generated by the oxidation of these metabolites [24–26]. However, unlike exopolysaccharide, PHB is an intracellular product and therefore additionally provides a means of storing excess carbon and reducing power for future use [27]. In this context, it is interesting to note that *Pseudomonas aeruginosa* can synthesize PHB or other Polyhydroxyalkanoates, exopolysaccharide, and/or various organic acids as alternative products, after losing its ability to make exopolysaccharide or PHB, respectively, by following natural strain degeneration or mutagenesis [25, 28].

3.5. Effect of Different Carbon Sources on PHB Production. The yield of PHB based on various carbon sources was studied and the values along with standard deviation is tabulated in Table 2. When the carbon source, mannitol, in the YEM medium was replaced by molasses, a maximum yield of 70% PHB was obtained. This research finding was a success as the molasses were used and the cost due to the use of mannitol can also be avoided. This is an economical initiative of using molasses as a source of carbon in the growth medium of *Pseudomonas aeruginosa*.

The Dunnett's multiple comparison test was used to find the statistical significance of the various carbon sources in comparison with the control (YEM). In Table 3, the values

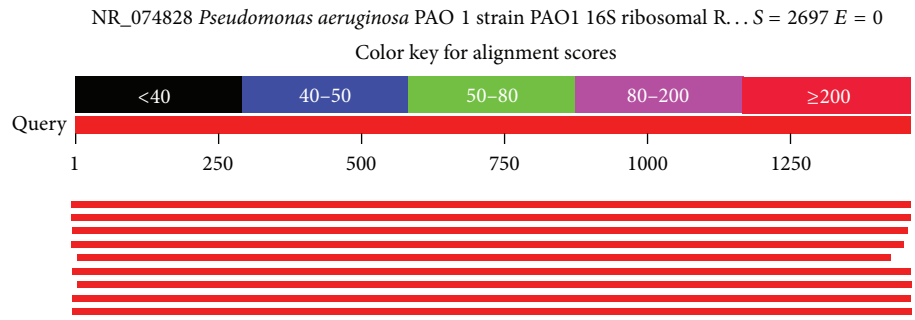

Figure 5: BLAST results for the isolated bacterial strain *Pseudomonas aeruginosa*.

Table 1: Biochemical and morphological characterization of isolated bacterial strain.

Biochemical test	Result	Morphology	Result
Gram staining	−	Colony shape	Irregular round
Catalase test	+	Colony elevation	Flat
Oxidase test	+	Colony size (mm)	2.5
Indole test	−	Colony margin	Serrated
Phenol red test	−	Colony color	Dull white
Methyl red test	−	Motility	Motile
Growth on EMB agar	−	Cell shape	Rod
Growth on MacConkey agar	−		

Table 2: Effect of various carbon sources on PHB production with standard deviation values.

Carbon source	Dry cell weight (g/L)	SD	Amount of PHB (g/L)	SD	PHB yield (%)	SD
Glucose	0.22	0.007	0.026	0.0007	11.82	0.049
Sucrose	0.23	0.01	0.049	0.0008	21.30	0.141
Fructose	1.32	0.0282	0.072	0.0021	5.45	0.219
Molasses	0.21	0.0131	0.147	0.0014	70.0	0.707
Control (YEM)	0.28	0.0141	0.060	0.0135	21.43	0.0636

SD: standard deviation.

Table 3: Effect of various carbon sources on PHB production with standard deviation values.

Dunnett's multiple comparison test	Significant	Summary	Adjusted p value
	Dry cell weight		
Control (YEM) versus glucose	Yes	*	0.0178
Control (YEM) versus sucrose	No	ns	0.0683
Control (YEM) versus fructose	Yes	****	<0.0001
Control (YEM) versus molasses	Yes	*	0.0235
	Amount of PHB		
Control (YEM) versus glucose	Yes	**	0.0029
Control (YEM) versus sucrose	No	ns	0.0682
Control (YEM) versus fructose	No	ns	0.9459
Control (YEM) versus molasses	Yes	***	0.0002
	% yield of PHB		
Control (YEM) versus glucose	Yes	****	<0.0001
Control (YEM) versus sucrose	No	ns	>0.9999
Control (YEM) versus fructose	Yes	****	<0.0001
Control (YEM) versus molasses	Yes	****	<0.0001

ns: not significant.

with $p < 0.05$ are considered significant with symbol $*$ indicating mild significance and symbol $* * **$ indicating more significance in comparison with the control medium.

3.6. Effect of Time on PHB Production. It was found that when molasses were used as the carbon source in YEM medium instead of mannitol, at the end of 48 hours, the PHB yield was 70%. After 48 hours of incubation, there was a decrease in the PHB yield and increase in the viscosity of the medium. The increase in the viscosity of the growth medium resulted in a limited oxygen transfer rate and caused the fall of PHB synthesis and accumulation inside the bacterial cells. The PHB yield decreased to 32% after 72 hours of incubation and 18% after 120 hours of incubation. Even the dry cell weight was increased up to 120 hours. The decrease in the PHB content explained that the bacteria have used the produced PHB as a source of carbon to survive due to the unavailability of the carbon source. The %PHB yield along with the standard deviation values has been plotted as shown in Figure 6.

3.7. Spent Wash Decolorization Study. This initiative of testing the effect of the isolated bacterial culture and the as-synthesized PHB on spent wash decolorization was performed as a trial and very positive and welcoming results were obtained as shown in Figure 7. At the end of five-day batch study, a combination consisting of 2 mL (1 : 1 ratio) of PHB and *Pseudomonas aeruginosa* culture was able to achieve 92.77% spent wash color removal, whereas there was only a minimal color reduction (25.30% and 13.58%) resulting when the spent wash was treated with microorganism and PHB individually. The increased color removal was due to the phenomenon that the Gram-negative bacteria, *Pseudomonas aeruginosa,* possess negative surface charge. When these bacterial cultures were added to the effluent along with the PHB, the bacteria bind to the PHB, hence forming a biofilm, and thereby also act as an ion exchange that attracts the suspended organic particles to get bound to the biofilm. This biofilm acts as a support material and favors a suitable condition for the further growth and development of the bacteria. Thus, the synergic actions of the PHB and the microorganism were found to be the most capable of performing spent wash decolorization.

3.8. Research Outcome. The positive results of this research could lead to a more advanced technique and application where the microbially produced PHB can be used as a nanobiomaterial possessing tunable properties with a focused application for binding and removal of heavy metals from aqueous industrial effluents. As the synthesis of biopolymer relies on a principle of single phase transition, scaling up the production process could be of a less intensive task, hence providing an ecofriendly technology for pollutant removal.

4. Conclusion

In this research paper, bacterial strain possessing tolerance to high concentrations of chromium was isolated from tannery effluent. The isolated strain was identified as *Pseudomonas aeruginosa* by biochemical and molecular characterization.

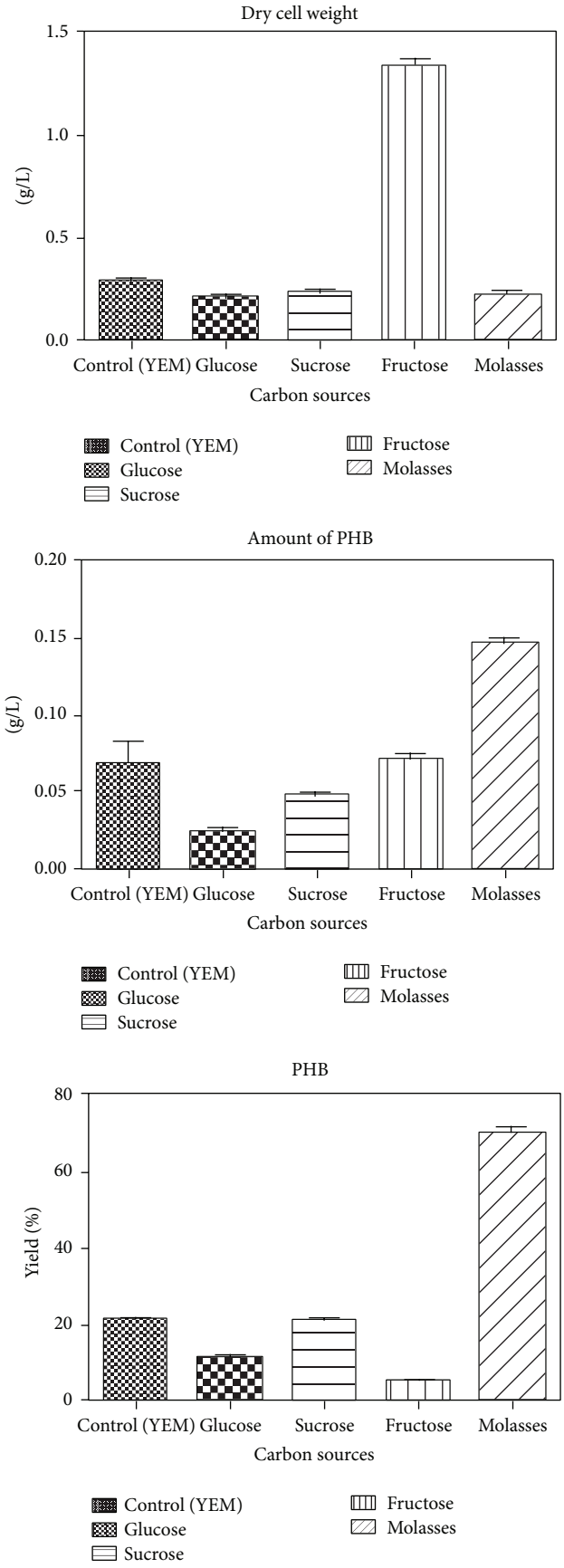

Figure 6: Bar charts with standard deviation values for dry cell weight, amount of PHB, and % yield of PHB.

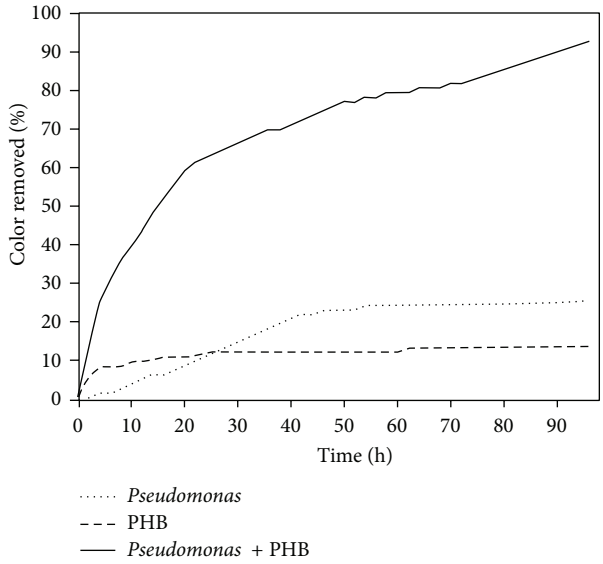

Figure 7: Effect of time on % color removal by microbial culture, PHB, and combination of microbial culture and PHB.

Efforts were taken to synthesize Polyhydroxybutyrate (PHB) using the isolated bacterial strain. Mannitol, an expensive carbon source for the bacterial growth culture media, was replaced with inexpensive molasses. The exopolysaccharides accumulated by the bacterial cells were harvested and separated. Optimization of suitable quantities of as-synthesized PHB and microbial culture was tested to evaluate the color removal efficiency. The results showed that *Pseudomonas aeruginosa* exhibited a synergistic effect in combination (1 : 1 ratio) with the biopolymer towards spent wash decolorization. Lab scale optimization experiments resulted in 92.77% removal of spent wash color after 96 hours of treatment, whereas there was only a limited color reduction (25.30% and 13.58%) observed when the same concentration and volume of spent wash was treated with *Pseudomonas aeruginosa* culture and PHB individually.

Conflict of Interests

The authors report no conflict of interests.

Acknowledgments

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Research Article

Characteristics of Biological Nitrogen Removal in a Multiple Anoxic and Aerobic Biological Nutrient Removal Process

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Two sequencing batch reactors, one with the conventional anoxic and aerobic (AO) process and the other with the multiple AO process, were operated to examine characteristics of biological nitrogen removal, especially of the multiple AO process. The long-term operation showed that the total nitrogen removal percentage of the multiple AO reactor was 38.7% higher than that of the AO reactor. In the multiple AO reactor, at the initial SBR cycle stage, due to the occurrence of simultaneous nitrification and denitrification, no nitrite and/or nitrate were accumulated. In the multiple AO reactor, activities of nitrite oxidizing bacteria were inhibited due to the multiple AO operating mode applied, resulting in the partial nitrification. Denitrifiers in the multiple AO reactor mainly utilized internal organic carbon for denitrification, and their activities were lower than those of denitrifiers in the AO reactor utilizing external organic carbon.

1. Introduction

Nitrogen and phosphorus in discharged wastewater can be key inducers for the eutrophication of receiving water bodies. As a protective environmental strategy, stringent nitrogen and phosphorus discharge standards from wastewater have been set in many countries, such as concentrations below 3 mg/L for total nitrogen (TN) and below 0.1 mg/L for total phosphorus (TP) in some USA areas [1]. Consequently, it is necessary to develop new or optimize the existing wastewater treatment technologies for compliance with the latest discharge standards.

Biological nitrogen removal is achieved by sequential nitrification under aerobic conditions and denitrification under anoxic conditions. During nitrification, ammonium is oxidized to nitrite by ammonium oxidizing bacteria (AOB) and then to nitrate by nitrite oxidizing bacteria (NOB). During denitrification, nitrite and/or nitrate is denitrified to nitrogen gas with organic carbon as the electron donor. Usually, predenitrification is widely applied for biological nitrogen removal, where denitrification occurs firstly in the anoxic phase by recycling nitrified wastewater from the following aerobic phase. In this type of anoxic and aerobic (AO) process, removal percentage of TN depends on the recycling ratio and organic carbon supplied. In order to achieve a high TN removal percentage, the recycling ratio should be increased and adequate organic carbon should be provided for complete denitrification. However, a high recycling ratio will bring the dissolved oxygen (DO) from the aerobic phase to the anoxic phase, reducing the organic carbon available for denitrification. In addition, in the conventional AO process, competition between denitrifiers and polyphosphate accumulating organisms (PAOs) also occurs and it is very difficult to achieve high removal efficiencies for TN and TP simultaneously [2]. Sometimes, postdenitrification is also adopted through endogenous respiration of heterotrophs for denitrification of the oxidized nitrogen, but the reaction rate of this process is relatively slow and requires long reaction duration. Therefore, postdenitrification by the addition of external organic carbons to enhance denitrification can be applied to enhance biological nitrogen removal, but this proves impeditive to the operational cost of wastewater treatment plants.

The multiple AO process is developed by optimizing the AO process, where intermittent aeration is adopted to achieve multiple nitrification and denitrification stages within one

reaction phase in sequencing batch reactors (SBRs) or within one reaction zone in constant flow reactors. By this means, several advantages for improving biological nitrogen removal could be realized. Firstly, recycling mixed liquor between aerobic and anoxic phases will be reduced or omitted, resulting in a low concentration of DO recycled back to the anoxic phase and a low energy cost for recycling mixed liquor. Secondly, the competition between denitrifiers and PAOs is also alleviated, which can enhance phosphorus release during the anaerobic phase. Finally, alkalinity increases during denitrification in the anoxic phase can compensate for its reduction during nitrification in the aerobic phase, which can stabilize pH in the system and maintain high activities for both nitrifiers and denitrifiers [3, 4].

In the multiple AO process, when switching from the anoxic phase to the aerobic phase, nitrite accumulation often occurs due to the longer lag time of NOB than that of AOB, which may enhance shortcut nitrification and denitrification [5, 6]. By this means, not only nitrogen removal is improved, but also the aeration and organic carbon requirement is reduced [7–10]. In the multiple AO process, DO is an important factor affecting nitrification and denitrification efficiencies. Ruiz et al. [11] and Chuang et al. [12] found that, under low DO concentrations of 0.2–0.7 mg/L, partial nitrification could be easily achieved. While Ciudad et al. [13] found that, even under a DO concentration of 1.4 mg/L in activated sludge systems, nitrite accumulation still occurred, while in biofilm systems, Oyanedel-Craver et al. [14] found that nitrite accumulation even occurred with DO as high as 3.5 mg/L. Li et al. [2] found that, by using the intermittently operating mode, nitrite accumulation with a ratio to total oxidized nitrogen of above 64% could be achieved for DO in the range of 0.7–6 mg/L. From previous studies, different floc sizes and biofilm thicknesses might induce different microenvironmental conditions for the different nitrite accumulation phenomena [15]. Until now, characteristics of biological nitrogen removal in the multiple AO process are still not clear and further researches are required.

In this study, two SBRs, one with the conventional AO operating mode and the other with the multiple AO operating mode, were operated in the lab to examine characteristics of biological nitrogen removal. The conditions examined included long-term system operation, typical SBR cycles, and batch experiments for the examination of activities of both nitrification and denitrification. The research outputs would provide some new knowledge for advancing the multiple AO technology for enhanced biological nitrogen removal.

2. Materials and Methods

2.1. System Operation. Two parallel lab-scale SBRs, one of the common AO process and the other of the multiple AO process, were operated at 25°C. The working volume of the SBRs was 8 L. Each 24-hour period included 4 reaction cycles, and each cycle was 6 hours. The operation cycle of the AO SBR was 120 min anaerobic phase (including 10 min filling), 180 min aerobic phase, and settlement and withdrawal of treated wastewater of 60 min. The operation cycle of the multiple AO SBR was 120 min anaerobic phase (including 10 min filling), 30 min aerobic phase, 30 min anoxic phase, 30 min aerobic phase, 30 min anoxic phase, 60 min aerobic phase, and settlement and withdrawal of treated wastewater of 60 min. According to the study of Li et al. [2], the alternative anoxic phase of 30 min and aerobic phase of 30 min were able to inhibit the activities of NOB. The operations of the SBRs were controlled by timers for filling, mixing, aeration, and withdrawal. During aerobic phases, aeration was achieved through air pumps with micropore stones and the temperature was controlled by a heater. During anoxic or anaerobic phases, aeration was stopped and the reactors were stirred only by magnetic stirrers.

Each cycle, 4 litres of treated wastewater (3.5 L during the sludge discharging cycle) was discharged and 4 litres of influent wastewater was pumped into the reactors by peristaltic pumps. For each 24-hour period, during the final aerobic phase just before the settlement, 0.5 litres of mixed liquor was removed from both reactors to control the sludge retention time of around 16 days. The AO reactor was seeded with activated sludge taken from Nanshan Wastewater Treatment Plant, Shenzhen, China, while the multiple AO reactor was seeded with activated sludge taken from the AO reactor after more than three months of operation of the AO SBR.

The influent wastewater was made from the following components: 510 mg/L sodium acetate (NaAc), 153 mg/L NH_4Cl, 14 mg/L $CaCl_2{\cdot}2H_2O$, 90 mg/L $MgSO_4{\cdot}7H_2O$, 46 mg/L Na_2HPO_4, 10 mg/L yeast extract, 200 mg/L $NaHCO_3$, and 0.4 mL/L trace elements. The components of the trace elements were added according to Smolders et al. [16]. The influent wastewater contained the chemical oxygen demand (COD) concentration of around 400 mg/L, the ammonium nitrogen (NH_4-N) concentration of around 40 mg/L, and the orthophosphate (PO_4-P) concentration of around 10 mg/L.

2.2. Batch Experiments. For activated sludge under steady state, batch experiments were carried out to examine activities of nitrification and denitrification for activated sludge acclimated in the AO reactor and the multiple AO reactor. Average results of replications for each experiment were presented.

Batch nitrification experiments were carried out as follows. (1) Activated sludge was taken from the AO reactor and the multiple AO reactor just before the end of the last aerobic phase and centrifuged at 12000 rpm for 2 min, and then the supernatant was discarded. (2) The activated sludge was resuspended using the synthetic wastewater but without the addition of ammonium and acetate, and samples were taken for suspended solids (SS) and volatile suspended solids (VSS) measurement. (3) Ammonium was added to the mixed liquor from each reactor with the initial NH_4-N concentration of 30 mg/L. (4) Batch nitrification experiments were started by aeration, and samples were taken at intervals of 10–15 min. NH_4-N, nitrite nitrogen (NO_2-N), and nitrate nitrogen (NO_3-N) were measured and nitrification activities were obtained by linear regression of these parameters with time.

For batch denitrification experiments with the external organic carbon as the electron donor, after taking the activated sludge from the two SBRs before the end of the aerobic phase, acetate and nitrate were added to the mixed liquor

Table 1: Influent and effluent water quality from the AO and multiple AO reactors (unit of mg/L).

	Influent	Effluent from the AO reactor	Effluent from the multiple AO reactor
COD	374	32.9	39.4
NH_4-N	38.3	0.06	0.02
NO_2-N	—	0.02	0.11
NO_3-N	—	12.3	1.2
TN	—	12.3	2.2
PO_4-P	9.8	0.26	0.25
TP	—	0.42	0.76

with an initial concentration of 500 mg/L for sodium acetate and 30 mg/L for NO_3-N. The batch reactors with the mixed liquor were sealed and mixed using magnetic stirrers, and the experiment was started. Samples were taken at intervals of 10–15 min and parameters of NO_2-N, NO_3-N, PO_4-P, acetate, and polyhydroxybutyrate (PHB) were measured.

For batch denitrification experiments with the internal organic carbon as the electron donor, after taking the activated sludge from the two SBRs just before the end of the anaerobic phase, nitrite and nitrate were added to the mixed liquor with an initial concentration of 10 mg/L for NO_2-N and 30 mg/L for NO_3-N. The batch reactors with the mixed liquor were sealed and mixed using magnetic stirrers, and the experiment was started. Samples were taken at intervals of 10–15 min and parameters of NO_2-N, NO_3-N, and PHB were measured.

For batch denitrification experiments under organic carbon limited conditions, after taking the activated sludge from the two SBRs before the end of the aerobic phase (without external easily biodegradable organic carbon and limited internal organic carbon), ammonium and nitrite were added to the mixed liquor with an initial concentration of 10 mg/L for NH_4-N of and 10 mg/L for NO_2-N. The batch reactors with the mixed liquor were sealed and mixed using magnetic stirrers, and the experiment was started. Samples were taken at 10 min intervals and parameters of NH_4-N, NO_2-N, and NO_3-N were measured.

2.3. Analytical Methods. COD, TN, TP, NH_4-N, NO_3-N, NO_2-N, PO_4-P, SS, and VSS were tested according to standard methods for the examination of water and wastewater [17]. pH and DO were measured using WTW portable pH meter (pH 3110, WTW, Germany) and DO meter (Oxi 315i, WTW, Germany), respectively.

PHB was tested according to the method of Karr et al. [18] and Rodgers and Wu [19]. Mixed liquor of 2 mL was taken from the SBRs and centrifuged at 12000 rpm for 2 min and the supernatant was discarded. The sludge was sequentially dewatered by 50%, 80%, and 96% ethanol solutions, each for 3 min, and then centrifuged at 12000 rpm for 2 min. After dewatering, the sludge was transferred to a glass tube with concentrated sulfuric acid twice, each time with a volume of 0.5 mL. The mixed liquor with concentrated sulfuric acid was digested at 100°C for 30 min and shaken every 10 min to mix the sample thoroughly. After digestion, 4 mL of deionized water was added to the glass tube, mixed, cooled to room temperature, and centrifuged at 12000 rpm for 2 min. The supernatant was then ready for testing of PHB with the HPLC (Shimadzu LC-20A, Japan). The HPLC had a UV detector at 210 nm and an Aminex HPLC Organic Acid Analysis Column (HPX-87H, Bio-Rad, USA). The mobile phase was 1‰ sulfuric acid with the flow rate of 0.6 mL/min. Acetate was also tested by HPLC with the procedure as those of the PHB testing.

3. Results and Discussion

3.1. Long-Term System Operation. After more than three months of operation, the system performance under steady state is shown in Table 1.

The average SS concentration was 3.16 g/L in the AO reactor and 3.68 g/L in the multiple AO reactor, with the VSS/SS ratio of 83% in both reactors. The effluent SS was 5.2 mg/L in the AO reactor and 9.0 mg/L in the multiple AO reactor. The measured synthetic wastewater contained concentrations for COD of 374 mg/L, NH_4-N of 38.3 mg/L, and PO_4-P of 9.8 mg/L. Both reactors removed COD, NH_4-N, TN, and TP efficiently, with their removal percentages of 90.8%, 99.8%, 67.9%, and 95.7% in the AO reactor and 88.4%, 100%, 94.2%, and 92.2% in the multiple AO reactor. For the filtered and unfiltered effluent, there was not much difference in COD, TN, and TP concentrations due to the low effluent SS concentration (below 10 mg/L) in both reactors.

3.2. Typical SBR Cycle Performance. For a typical SBR cycle, the dynamics of nitrogen, phosphorus, pH, and DO in the two reactors are shown in Figure 1.

In the AO reactor, during a typical cycle, ammonium increased during the fill step and peaked at the end of the fill step; its concentration remained stable during the anaerobic phase and then nitrified to nitrite and nitrate during the aerobic phase. Nitrite was accumulated during the initial aerobic phase and then decreased with further nitrification. Nitrate denitrified quickly during the fill step and then produced during nitrification in the aerobic phase with the concentration reaching around 15.9 mg/L. During the anaerobic phase, PAOs released the phosphorus and the phosphorus concentration reached to 86.4 mg/L after 2 h anaerobic reaction. During the aerobic phase, PO_4-P was taken up with a final concentration of less than 1.0 mg/L.

In the multiple AO reactor, during the typical cycle, similar phenomena were observed as those in the AO reactor. During the aerobic phase, NO_2-N was accumulated during the initial stage of the final aerobic phase with the maximum concentration reaching 1.6 mg/L. There was no obvious NO_3-N produced during the alternative anoxic and aerobic phases, while it increased during the final 1 hour aerobic phase and reached to 1.4 mg/L. For PAOs, PO_4-P was released during the anaerobic phase and then taken up during the aerobic phase, while it did not vary much during the anoxic phase.

In both reactors, the pH remained in the range of 7.5–8.5, which was benefit for the activities of nitrifiers or PAOs.

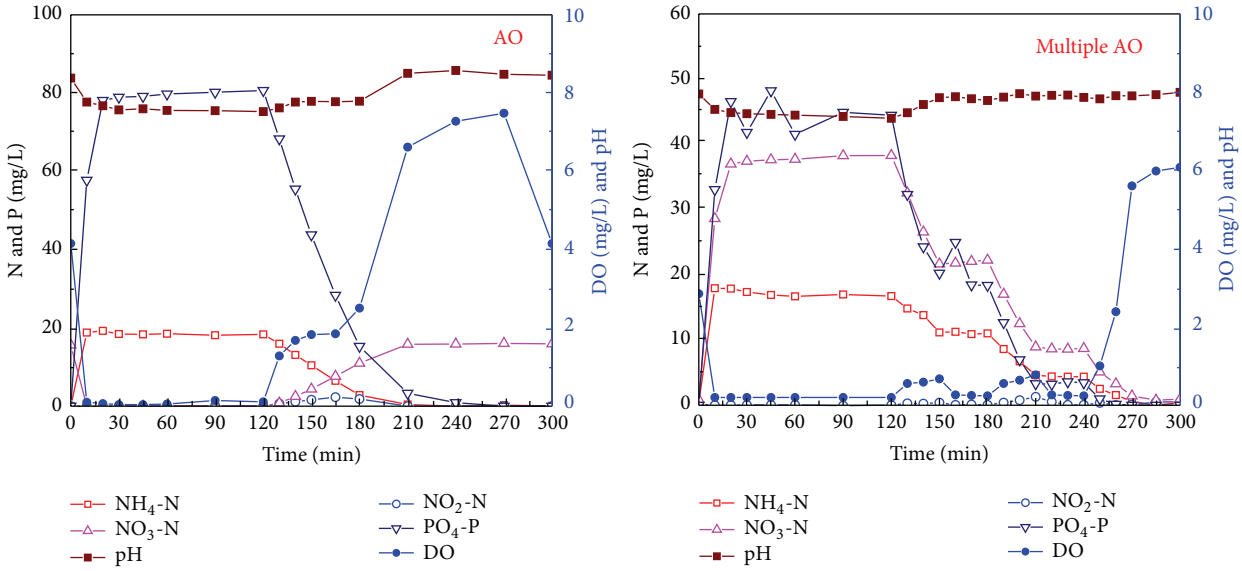

Figure 1: Dynamics of various parameters during typical cycles in the AO and multiple AO reactors.

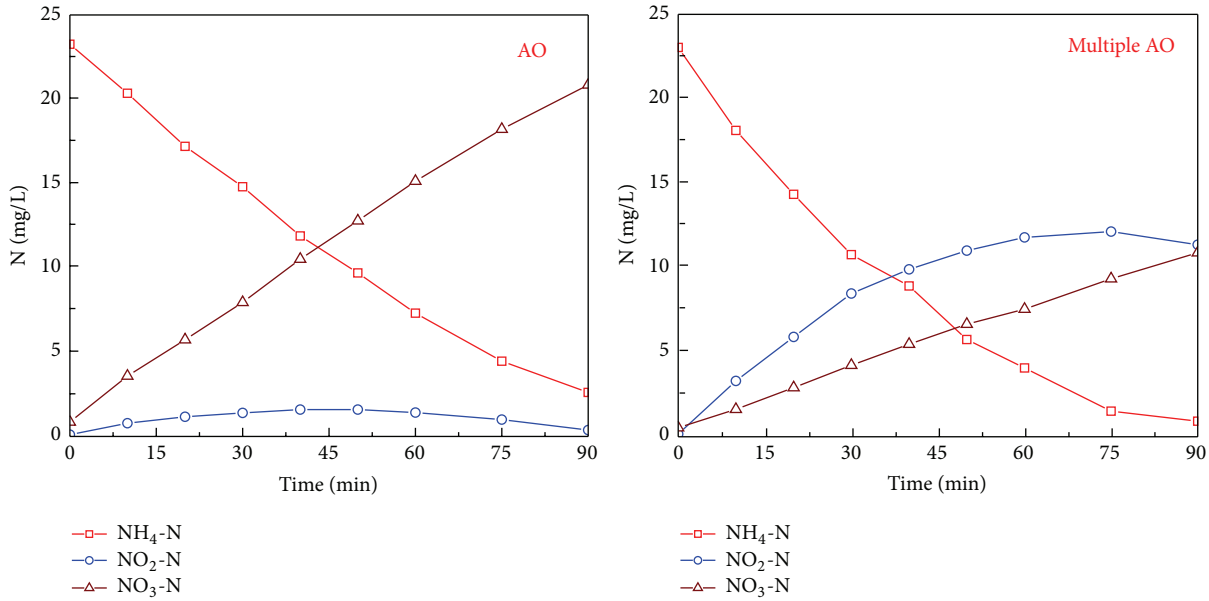

Figure 2: Dynamics of various types of nitrogen during the batch nitrification for activated sludge taken from both AO and multiple AO reactors.

During the aerobic phase, due to activities of nitrifiers and heterotrophs, DO increased slowly during the initial aerobic phase and reached to above 6 mg/L after 1 hour when ammonium was nitrified completely in the AO reactor. In the multiple AO reactor, DO concentrations remained low before the final aerobic phase, with concentrations below 1 mg/L, while during the final aerobic phase, DO concentration increased with the complete of nitrification and finally reached to around 5.7 mg/L.

3.3. Batch Nitrification Experiments. The batch nitrification experiment results for activated sludge taken from both the AO reactor and the multiple AO reactor are shown in Figure 2.

For activated sludge taken from the AO reactor, with the reduction of ammonium, nitrate production occurred with only a small amount of nitrite accumulated. The NH_4-N reduction rate was 6.6 mg N/g VSS/h and the NO_3-N production rate was 5.6 mg N/g VSS/h; NO_2-N accumulated during the initial 40 minutes, peaked at 1.6 mg/L, and then decreased to below 0.4 mg/L.

For activated sludge taken from the multiple AO reactor, the reduction rate of NH_4-N was 8.1 mg N/g VSS/h, and its concentration was below 1 mg/L after 90 minutes reaction.

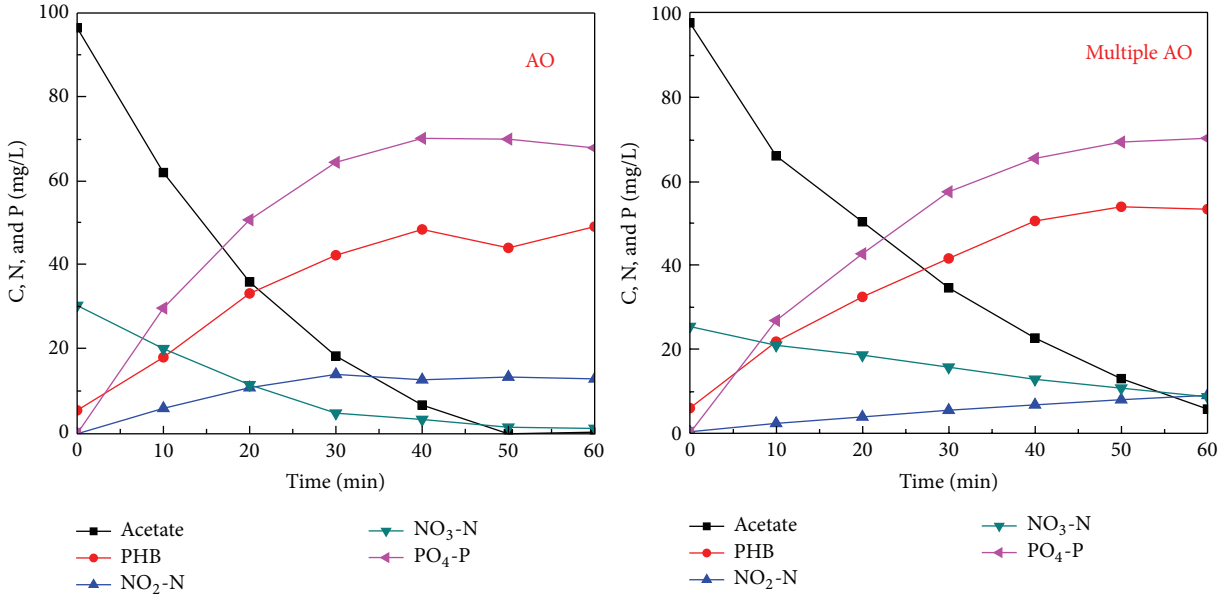

FIGURE 3: Dynamics of various types of nitrogen, organic carbon, and phosphorus during denitrification with external organic carbon as the electron donor for activated sludge taken from both AO and multiple AO reactors.

With the reduction of ammonium, accumulation of nitrite was expected, and its accumulation rate was 5.5 mg N/g VSS/h during the initial 30 minutes. The NO_2-N concentration peaked at 12.1 mg/L after 75 minutes reaction and then decreased thereafter. The NO_3-N production rate was 2.3 mg N/g VSS/h over the whole reaction phase.

3.4. Batch Denitrification Experiments. The results of batch denitrification experiments with the external organic carbon as the electron donor are shown in Figure 3. For activated sludge taken from the AO reactor, during the initial 30 minutes, with an adequate supply of acetate, the NO_3-N reduction rate was 31.9 mg N/g VSS/h and then decreased thereafter; accompanied with the reduction of nitrate, NO_2-N was accumulated with the accumulation rate of 17.7 mg N/g VSS/h during the initial 30 minutes, and the NO_2-N concentration was stable during the final 30 minutes with the consumption of acetate. For activated sludge taken from the multiple AO reactor, denitrification was carried out slowly with the external organic carbon as the electron donor, with a NO_3-N reduction rate of 10.2 mg N/g VSS/h and a nitrite accumulation rate of 5.3 mg N/g VSS/h. The acetate utilization rate was 96.9 mg C/g VSS/h in the AO reactor during the initial 30 minutes, and it was 54.6 mg C/g VSS/h in the multiple AO reactor over the whole reaction period. Acetate was partially stored as PHB and the PHB production rate was 46.9 mg/g VSS/h in the AO reactor during the initial 30 minutes and 40.6 mg/g VSS/h in the multiple AO reactor over the whole reaction period. During the initial 30 minutes, the PO_4-P release rate was 80 mg P/g VSS/h in the AO reactor and 70.2 mg P/g VSS/h in the multiple AO reactor.

The results of batch denitrification experiments with the internal organic carbon as the electron donor are shown in Figure 4. For activated sludge taken from the AO reactor, denitrification was slow, with the NO_3-N reduction rate of 2.2 mg N/g VSS/h, and the NO_2-N concentration remained relatively stable. For activated sludge taken from the multiple AO reactor, during the initial 30 minutes, the NO_3-N reduction rate was 5.6 mg N/g VSS/h and the NO_2-N production rate was 4.2 mg N/g VSS/h, while, during the latter 30 min, the NO_3-N reduction rate was 1.9 mg N/g VSS/h and the NO_2-N production rate was 1.2 mg N/g VSS/h.

The results of batch denitrification experiments during organic carbon limited conditions are shown in Figure 5. For activated sludge taken from the AO reactor, denitrification was very slow, and the NO_3-N reduction rate was 0.89 mg N/g VSS/h and the NO_2-N reduction rate was 0.4 mg N/g VSS/h. For activated sludge taken from the multiple AO reactor, the NO_3-N reduction rate was 0.78 mg N/g VSS/h and the NO_2-N reduction rate was 0.23 mg N/g VSS/h. Under all conditions, the concentration of ammonium was stable, indicating that no anaerobic ammonium oxidation occurred. The reduced oxidized nitrogen was mainly due to endogenous respiration.

4. Discussion

In the multiple AO technology, by adopting the multiple alternative AO process, simultaneous nitrification and denitrification could be enhanced and complete nitrification would occur in the final aerobic phase. In the present study, the multiple AO reactor shortened the aerobic phase by 33.3% compared with the AO reactor. Under steady state, the multiple AO reactor improved the TN removal percentage by 38.7% compared with the AO reactor. Therefore, the multiple AO process could improve TN removal significantly. Similar results were also obtained for TN removal from municipal wastewater and slaughterhouse wastewater by using the

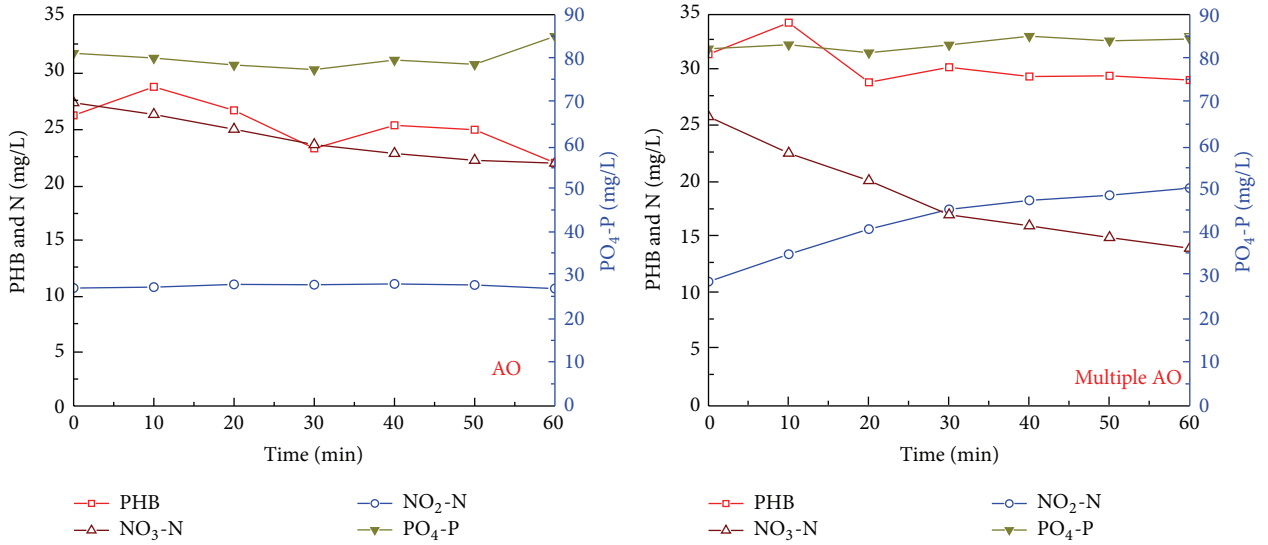

Figure 4: Dynamics of various types of nitrogen, PHB, and phosphorus during denitrification with internal organic carbon as the electron donor for activated sludge taken from both AO and multiple AO reactors.

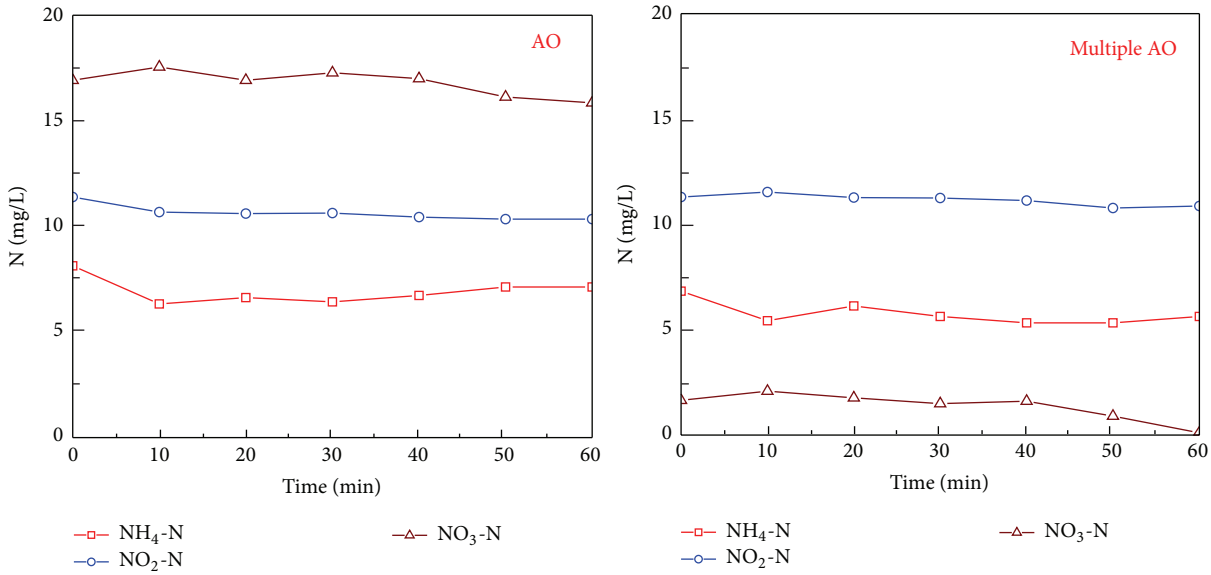

Figure 5: Dynamics of various types of nitrogen during denitrification under organic carbon limited conditions for activated sludge taken from both AO and multiple AO reactors.

multiple AO processes [2, 20]. Li et al. [2] obtained a TN removal percentage of 96% in an intermittently aerated SBR for treating high ammonium concentration slaughterhouse wastewater. Sasaki et al. [20] obtained a removal percentage of TN of 92% by using two-stage intermittently aerated reactors treating municipal wastewater.

During the initial SBR cycle, with the reduction of NH_4-N, no accumulation of both NO_2-N and NO_3-N was observed in the multiple AO reactor, indicating simultaneous nitrification and denitrification occurred inside the reactor [7]. By adopting an alternative anoxic phase of 30 min and aerobic phase of 30 min, no NO_3-N accumulation was observed during the initial 2 hours, and even during the first hour of the final aerobic phase, the NO_3-N concentration remained at a very low concentration of below 0.15 mg/L during the initial 30 min, which finally increased to above 1.4 mg/L during the final stage. Li et al. [2] and Zhang et al. [6] observed in their intermittently aerated systems that activities for NOB had recovered after 60 min and 16–18 min after recovering from the anoxic conditions. Therefore, the operating mode of an alternative anoxic and aerobic mode could induce nitrite accumulation and result in shortcut nitrification and denitrification.

From the batch nitrification experiment, a higher NH_4-N nitrification rate was observed in the multiple AO reactor than that in the AO reactor, while a low NO_2-N nitrification

rate was obtained from the multiple AO reactor. Therefore, a high nitrite accumulation potential and a peak NO_2-N concentration of 12.1 mg/L were obtained from the multiple AO reactor. These results showed that the multiple AO operating mode could inhibit the activities of NOB. In the multiple AO reactor, DO was in the range of 0.4–0.7 mg/L during the initial anoxic and aerobic alternative stage, which might also inhibit activities of NOB and resulted in the shortcut nitrification and denitrification [11, 12]. Therefore, in the multiple AO reactor, due to the low DO concentration and the alternative anoxic and aerobic operating mode, NOB activities were inhibited, and once nitrified NH_4-N to NO_2-N, denitrifiers could use NO_2-N directly for denitrification through the shortcut denitrification process [21, 22].

When the external organic carbon was used as the electron donor for denitrification, a high denitrification rate was obtained in the AO reactor, while when the internal organic carbon was used as the organic carbon, a high denitrification rate was obtained in the multiple AO reactor. The reason could be due to the fact that, in the multiple AO reactor, the organic carbon was mainly stored as the internal organic carbon and then utilized by both PAOs and denitrifiers. Therefore, the denitrification rate for denitrifiers from the multiple AO reactor was relatively low with the utilization of the internal organic carbon, because utilization of PHB usually limited the biological reactions as shown by some previous studies [23]. Zeng et al. [7] found that, in the anoxic/aerobic biological nitrogen and phosphorus removal systems, COD in the influent was mainly stored by PAOs or GAOs as polyhydroxyalkanoate (PHA) and then used later for phosphorus uptaken or denitrification. Mino et al. [24] and Smolders et al. [16] found that when acetate was used as the external organic carbon, it would be mainly stored as PHB. Similarly, in the present study, acetate would be also accumulated as PHB during the anaerobic phase and then used for denitrification during the alternative AO phases, which could improve the utilization efficiency of organic carbons and enhance biological nitrogen removal, such that a high TN removal percentage of 94.2% was obtained in this study.

In the multiple AO reactor, during the initial fill step, only a minor concentration of oxidized nitrogen existed inside the reactor and this also inhibited the acclimation of denitrifiers which could utilize the external organic carbon. This was also confirmed from the batch denitrification experiments. For activated sludge taken from the multiple AO reactor, the denitrifying rate only decreased from around 10.2 mg N/g VSS/h using external organic carbon to 5.6 mg N/g VSS/h using internal organic carbon, indicating that a high proportion of denitrifiers using internal organic carbons was acclimated in this reactor. While in the AO reactor, during the fill and anaerobic phase, denitrifiers which could utilize external organic carbon for denitrifying oxidized nitrogen remaining from the previous cycle were acclimated. This was also confirmed from the batch denitrification experiments. For activated sludge taken from the AO reactor, the denitrifying rate was decreased from around 31.9 mg N/g VSS/h using external organic carbon to 2.2 mg N/g VSS/h using internal organic carbon, indicating that a high proportion of denitrifiers using external organic carbons was acclimated in this reactor. Therefore, different types of denitrifiers might be acclimated in both reactors and this should be recognized when modelling denitrification with different operating modes.

5. Conclusions

(1) As to the influent COD of 374.4 mg/L, NH_4-N of 38.3 mg/L, and PO_4-P of 9.8 mg/L, the removal percentages of COD, NH_4-N, TN, and TP were 90.8%, 99.8%, 67.9%, and 95.7% in the AO reactor and were 88.4%, 99.9%, 94.2%, and 92.2% in the multiple AO reactor. In the multiple AO reactor, the TN removal percentage increased by 38.7% by adopting alternative anoxic and aerobic phases. (2) In the multiple AO reactor, during the initial alternative anoxic and aerobic phase, due to simultaneous nitrification and denitrification, no obvious nitrite or nitrate accumulation was observed. (3) In the multiple AO reactor, due to the applied alternative anoxic and aerobic mode and low DO concentrations during the initial aerobic phase, NOB was inhibited and a low nitrite nitrification rate was obtained. (4) Denitrifiers in the multiple AO reactor mainly utilized internal organic carbon as the electron donor for denitrification and its denitrifying activities were lower than those in the AO reactor where denitrifiers mainly utilized external organic carbon.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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