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Variable distributional characteristics of substrate utilization patterns in activated sludge plants in Kuwait

N. Z. Al-Mutairi *

Civil Engineering Department, Kuwait University, P.O. Box 5969, Safat 13060, Kuwait

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ABSTRACT

The objective of this study was to determine the magnitude of microbial functional potential and community structure between three different WWTPs using the Lorenz curve method and to find the effect of seasonal variation on patterns of substrate utilization. Lorenz curve method was sensitive enough to detect short-term changes in microbial functional diversity between Riqqa, Umm Al-Haiman and Al-Jahra activated sludge systems and showed seasonal variations of the utilized carbon sources. Gini coefficient ranged from 0.21 to 0.8. Lorenz curves seemed particularly suitable to present microbial heterogeneity in term of inequality and to highlight the relative contribution of low-and high functional diversity for the three different types of mixed liquors. Correlation analysis of the experimental data show that the complement of the Gini coefficient was strongly and positively correlated with the Shannon index (r_{xy} = 0.89), evenness (r_{xy} = 0.91), and AWCD (r_{xy} = 0.95) at the 95% level of significance (α = 0.05).

1. Introduction

Effluent standards for wastewater treatment plant (WWTP) are becoming more and more stringent (UNEP, 1999). To achieve these strict standards, at minimum costs, a better understanding of the ecological engineering approach is necessary to analyze the spatial and temporal functional diversity of microbial communities. In a simple system designed for retaining a single functional group of bacteria, each member is significant to system function. Increasing the functional redundancy guarantees a pool of functional responses to the instability through time, therefore ensuring a functionally more stable performance (Von Canstein et al., 2002).

The meaning and significance of microbial functional diversity and its' relation to effluent quality is an intractable but important aspect to any biological system. It is a widely held opinion that understanding the microbial functional diversity is an important step for more efficient and reliable biological treatment systems such as wastewater treatment plants (Victorio et al., 1996). Providing means to analyse microbial functional diversity is therefore an important, although not trivial, goal.

Measures of diversity and evenness have been used in the past to study substrate utilisation patterns from natural microbial populations in order to make inferences from the magnitude of microbial functional diversity (Al-Mutairi, 2007). Numerous techniques have been recently proposed to gain information from the functioning of microbial communities (Muyzer et al., 1993; Schneider et al., 1998). One of these techniques is metabolic experimentation of wastewater treatment systems which are based on isolation and culture methods to characterize microbial communities. However, these methods are restrictive because only a minority of microorganisms are selected (Schneider et al., 1998). Therefore, incorrect conclusions concerning the function and structure of the microbial community in situ are consequently made.

Due to the limitation of conventional microbiological methods, a range of different published works have used Biolog, an advance biological technique, to observe the potential activity of microbial communities. The Biolog method is extensively used, and present the advantage of being simple to perform and moderately inexpensive. Past studies have establish that it can be used to determine the degree to which two communities differ from one another (Garland and Mills, 1991; Al-Mutairi, 2007). These studies have also verified that microbial communities generate habitat-specific and reproducible patterns of carbon source utilization and so the technique can be used to distinguish temporal and spatial differences among microbial communities.

Both Garland (1996) and Zak et al. (1994) have recommended the use of particular analytical approaches to assess the rate and pattern of color development in the Biolog microtiter plates. These approaches have been used to quantify functional diversity, either by determining the average well color development (AWCD) or by measuring the substrate richness and evenness (Garland and Mills, 1991; Zak et al., 1994; Guckert et al., 1996; Victorio et al., 1996; Kaiser et al., 1998; Al-Mutairi, 2007). Furthermore, metabolic fingerprinting using the Biolog redox technology has already been shown to be effective in distinguishing heterotrophic microbial

* Fax: +965 4817524.
E-mail address: nayef@civil.kuniv.edu.kw

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communities within wastewater treatment systems (Hench et al., 2004; Al-Mutairi, 2007).

Although the response from the Biolog plates depends upon growth of the organisms, the whole community is potentially profiled. Furthermore, due to the relatively short incubation periods the expressed phenotypes are being determined rather than the phenotypic potential of the community. Consequently, variations in metabolic profile with sampling time can also be ascribed to changes in environmental conditions.

Present research interest is now focused on the types of quantitative information that can be attained from the BIOLOG technique and how this information can be used to interpret the functional diversity of microbial communities (Garland and Mills, 1991; Al-Mutairi, 2007). Weiner and Thomas (1986) developed the use of the Lorenz curve and its derived measure of inequality, the Gini coefficient, to monitor changes in the concentration of biomass in a population subjected to environmental perturbations. It is an alternative approach to quantify substrate richness and evenness. Furthermore, Harch et al. (1997) found that Lorenz curves and Gini coefficients can be a sound tool to characterize and assess the magnitude of bacterial communities variation in soil environment, and could complements other major analytical tools.

In order to optimize the performance of biological nutrient removal, and to achieve the strict effluent standards, therefore, the use of advance microbial analytical techniques can be beneficial. In addition, modifications in management practices in WWTPs may precede detectable changes in microbial diversity of the mixed liquor and thus provide an early signal of improvement in degradation (Schneider et al., 1998). Determination of this information could present the logic to further enhance the efficiency of wastewater treatment process. While it is imperative to characterize and identify the bacterial types involved in the secondary treatment process, Lorenz curve method would offer a more sensitive and ecologically significant measure of heterotrophic community structure.

The primary objective of this study was to analyze the magnitude of microbial functional diversity and community structure between three different WWTPs using the Lorenz curves and Gini coefficient. The second objective was to determine the effect of seasonal variation on patterns of microbial activities on the GN-Biolog plates. In addition, this study tries to explore opportunities to develop methodological and theoretical interfaces with microbial diversity of wastewater environment and their role in biological process.

2. Plants description

The three wastewater treatment plants understudy are owned and operated by the Ministry of Public Works (MPW). Umm Al-Haiman WWTP is the smallest and newest treatment plant in Kuwait, dating back to May 2001. It was constructed with a capacity of 27000 m^3/d of sewage, with a removal of 10500 kgBOD_5/d and 7800 kgTSS/d. Because carbonaceous BOD removal, nitrification, and denitrification can be accomplished within a single reactor, the process can produce a high quality effluent.

The Riqqa WWTP, in the other hand, was constructed in 1981 with a capacity of 180000 m^3/d. It was designed as a conventional aeration activated sludge system. Finally, the Jahra WWTP was constructed in the late 1981 with a capacity of about 70000 m^3/d. Jahra WWTP was treating a combined domestic and industrial wastewater of about 60000 m^3/d. Table 1 present operational values of the three government WWTPs under investigation. Table 1 also indicates that Riqqa and Umm Al-Haiman treatment plants have the highest organic removal efficiency (97%) compared with Al-Jahra treatment plant (90%).

3. Methods

3.1. Biolog Experiment

Duplicate wastewater samples were collected every two weeks from the three WWTPs over a period of one year. Mixed liquor grab samples (1 liter) were drawn from the aeration tank. All samples were collected in sterile screw-capped plastic bottles and samples were transported to the laboratory and stored at 4 °C prior to analysis. Sampling was conducted during the summer season period during the months of May–August and the winter season period during the months of December to February in 2003–2004.

Community level physiological profiling were used in order to determine the differences in microbial functional diversity. These plates were developed mainly for bacterial community analysis of environmental samples (Konopka et al., 1998) and have previously proven useful for pinpointing differences in microbial activity and community structure between wastewater treatment plants (Victorio et al., 1996; Kaiser et al., 1998; Al-Mutairi, 2007). The Biolog system (Biolog Inc., Hayward, CA) is based on the inoculation of 96-well microtiter plates with bacteria from environmental samples. Each well contains a tetrazolium dye and a carbon source that is unique to the well and serves as a basal nutrient medium, which may or may not support microbial growth. If growth occurs, the tetrazolium dye is reduced during the oxidation of the carbon source and the subsequent color change can be measured with a spectrophotometer (Garland and Mills, 1991). Biolog GN (Gram-negative) microtiter plates were directly inoculated with 100 μl aliquots of wastewater into each well according to the methods of Guckert et al. (1996).

Microorganisms were removed from the mixed liquor by homogenization for 1 min in a waring blender (Eberbach Model 34BL97), centrifuged for 5 min at 700 g, and the supernatant microbial fractions were washed three times with saline (0.85% NaCl) to remove contaminated organic material adhering to the cell's surface and present in the liquid phase. To eliminate the

Table 1
Operation information of the three WWTPs in Kuwait

Parameter	Wastewater treatment plants		
	Riqqa	Al-Jahra	Umm-Al-Haiman
Secondary treatment	Conventional aeration with fine bubble diffusers	Extended aeration with surface aerators	Oxidation ditch with mechanical aerators (rotors)
Design capacity (m^3/d)	180000	70000	27000
Actual flow (m^3/d)	145000	60000	12300
SRT (days)	5	30	10
F/M	0.5	0.1	0.2
MLSS (mg/L)	2000	4200	1500
Type of waste	Municipal and industrial	Municipal and industrial	Only municipal
Influent COD (mg/L)	300 ± 10	310 ± 40	430 ± 20
Effluent COD (mg/L)	9 ± 2	29 ± 5	13 ± 2
Date commissioned	1981	1981	2000