Talanta 125 (2014) 293–300

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Ubiquity of activated sludge ferricyanide-mediated BOD methods: A comparison of sludge seeds across wastewater treatment plants

Mark A. Jordan [a,b], David T. Welsh [a,*], Peter R. Teasdale [a]

[a] Environmental Futures Centre, Griffith University, Gold Coast Campus, Qld 4222, Australia
[b] School of Environment, Griffith University, Gold Coast Campus, Qld 4222, Australia

ARTICLE INFO

Article history:
Received 16 October 2013
Received in revised form
26 February 2014
Accepted 5 March 2014
Available online 17 March 2014

Keywords:
Biochemical oxygen demand
Trade waste
Sewage wastewater
Treated effluent
Environmental science
Analytical chemistry

ABSTRACT

Many studies have described alternatives to the BOD_5 standard method, with substantial decreases in incubation time observed. However, most of these have not maintained the features that make the BOD_5 assay so relevant – a high level of substrate bio-oxidation and use of wastewater treatment plant (WWTP) sludge as the biocatalyst. Two recently described ferricyanide-mediated (FM)-BOD assays, one for trade wastes and one for WWTP influents and treated effluents, satisfy these criteria and were investigated further here for their suitability for use with diverse biocatalysts. Both FM-BOD assays responded proportionately to increasing substrate concentration with sludges from 11 different WWTPs and temporally (months to years) using sludges from a single WWTP, confirming the broad applicability of both assays. Sludges from four WWTPs were selected as biocatalysts for each FM-BOD assay to compare FM-BOD equivalent values with BOD_5 (three different sludge seeds) measurements for 12 real wastewater samples (six per assay). Strong and significant relationships were established for both FM-BOD assays. This study has demonstrated that sludge sourced from many WWTPs may be used as the biocatalyst in either FM-BOD assay, as it is in the BOD_5 assay. The industry potential of these findings is substantial given the widespread use of the BOD_5 assay, the dramatically decreased incubation period (3–6 h) and the superior analytical range of both assays compared to the standard BOD_5 assay.

1. Introduction

Many studies have described fast alternatives to the BOD_5 standard method. Several approaches have shown promise, with great improvements in incubation time and analytical range, but they have not maintained the features that make the BOD_5 assay so relevant – a high level of substrate bio-oxidation and use of wastewater treatment plant (WWTP) sludge as the biocatalyst (see [1] and references therein). Ferricyanide-mediated (FM)-BOD assays boast high levels of substrate oxidation over incubation periods as little as 1 h [2,3]. The relevance and representativeness of FM-BOD measurements have since been improved by incorporating multi-species consortia as the biocatalyst [4,5]. However, WWTP activated sludge had not been successfully employed as biocatalyst until recently [1,6]. Largely due to use of activated sludge biocatalysts, significant and strong relationships were achieved between BOD_5 and FM-BOD measurements for a range of real wastewater samples: $n=35$, slope=1.07, $R=0.95$ using return activated sludge (RAS) as the biocatalyst with industrial wastewaters [6] and $n=33$, slope=0.94, $R=0.96$ using primary influent sludge (PIS) as the biocatalyst [1] for a mixture of WWTP influent, treated effluent, and greywater samples. Additionally, FM-BOD equivalent concentrations were determined within a single working day using both assays [1,6]. In the RAS FM-BOD assay, the biocatalyst was highly concentrated to maximize the analytical range and to achieve maximal substrate oxidation for the analysis of trade waste samples, which vary enormously in terms of composition, biodegradable complexity and BOD concentration. The PIS FM-BOD assay instead employed a much lower microbial concentration, to minimize the endogenous proportion of the FM-BOD measurement, in order to lower the limit of detection (LOD) of the assay to around that of the standard BOD_5 assay (2 mg BOD_5 L^{-1}) [7]. This made the more recently developed PIS FM-BOD assay of Jordan et al. [1] amenable to the measurement of low-range WWTP effluents and mid-range WWTP influents, the industrial application that the standard BOD_5 assay was principally designed for.

Abbreviations: APHA, American Public Health Association; BOD, biochemical oxygen demand; BOD_5, 5-day biochemical oxygen demand assay; FM, ferricyanide mediated; FM-BOD, ferricyanide mediated biochemical oxygen demand; GGA, glucose/glutamic acid; HACCP, hazard analysis and critical control points; LOD, limit of detection; MLSS, mixed liquor suspended solids; OD, optical density @ 600 nm; OECD, Organisation for Economic Cooperation and Development; PIS, primary influent sludge; RAS, return activated sludge; SSVI, stirred settled volume index; WWTP, wastewater treatment plant

* Corresponding author. Tel.: +61 7 55529186; fax: +61 7 55528067.
E-mail address: d.welsh@griffith.edu.au (D.T. Welsh).

http://dx.doi.org/10.1016/j.talanta.2014.03.004
0039-9140/

The metabolic activity of various sludges present in WWTPs is known to be inherently variable [8]. This is variability occurs both between WWTPs, given the considerable differences in bacterial diversity that arise due to differences in plant design and influent composition [9,10], and within plants, given intrinsic fluctuations of influent composition, environmental variables and manipulation of operating parameters over time. Despite this diversity in microbial assemblages and activities, the biological treatment component of all WWTPs is optimized to achieve efficient oxidation and assimilation of biodegradable organic carbon. The measure by which the carbonaceous removal efficiency of a WWTP is determined throughout the world is largely by means of the standard BOD_5 assay [7]. The BOD_5 assay typically incorporates activated sludge from a WWTP as the biocatalyst and therefore has to function well with the great diversity of sludges encountered. Therefore, to be effective and viable alternatives to this standard assay, new BOD methods must also be robust enough to allow sludges from a wide range of WWTPs to be utilized as the biocatalyst.

The principle aim of this study was to assess the ubiquity of application of the RAS and PIS FM-BOD bioassays by incorporating biocatalysts prepared from sludges collected from a number of WWTPs within the Gold Coast and Brisbane regions of southeast Queensland, Australia. It is anticipated that FM-BOD measurements will vary quantitatively from plant to plant and over time, along with sludge composition, but that when normalized against a known organic standard, calculated WWTP specific FM-BOD equivalent values should vary proportionately with BOD_5 values for a range of real wastewater samples. This would effectively demonstrate that both FM-BOD assays could be calibrated using a variety of sludge types and similar results could be obtained for the same wastewater samples using sludges from different WWTPs. This is an important step towards these bioassays being used widely within the wastewater industry, as the American Public Health Association (APHA) [7] states that alternative BOD assays may be employed where a proportionate relationship with the standard BOD_5 assay has been demonstrated.

2. Experimental

All reagents used in this study were of analytical grade and all dilutions were made using deionized (Milli-Q Element, Millipore) water. All reagents, samples and sludge biocatalysts were prepared according to the relevant assay [1,6]. For both assays, optimized conditions were used in this study and are summarized in Table 1.

2.1. Calculation of FM-BOD equivalent values

The concentration of microbially generated ferrocyanide was determined using chronoamperometry, as has been described previously [4,6], and is a measure of total FM-respiration. Net FM-respiration is determined by subtracting the limiting current for the control endogenous metabolism incubation from the sample/standard gross limiting current. Net respiration was used to calculate all FM-BOD equivalent values (see below).

Table 1
Summary of experimental parameters adopted in the RAS and PIS FM-BOD assays.

Exp. parameter	RAS FM-BOD [6]	PIS FM-BOD [1]
Biocatalyst	RAS	PIS
Pre-incubation conditions	Starved 24 h	Grown 24 h
Sludge conc. (OD)	10	0.25
Incubation time (h)	6	4

OD = optical density.

2.1.1. RAS FM-BOD bioassay [6]

FM-BOD equivalent values were determined according to Jordan et al. [6] where calibration data conformed to the Michaelis–Menten model. FM-BOD equivalent values were derived via a 3-point linear calibration according to Catterall et al. [4]. The OECD standard [11] was used to normalize most high range wastewater samples (i.e. >700 mg BOD_5 L^{-1}) and the GGA standard [7] for all mid-range wastewater samples (100–200 mg BOD_5 L^{-1}).

2.1.2. PIS FM-BOD bioassay [1]

FM-BOD equivalent values were derived from a 3-point linear calibration according to Catterall et al. [4]. The OECD standard [11] was used to normalize all moderate to high range wastewater samples (i.e. 10–500 mg BOD_5 L^{-1}) and the GGA standard [7] for all low-range wastewater samples (<10 mg BOD_5 L^{-1}).

2.2. Determining proportionality of FM-respiration responses with multiple sludges

2.2.1. Biocatalyst specifications

WWTP sludge (RAS and PIS) was collected from 11 different WWTPs in the southeast Queensland region. These WWTPs differed considerably in their capacities, design specifications, sludge concentrations (mixed liquor suspended solids (MLSS)), mean sludge age and sludge settleability (stirred settled volume index (SSVI)), and are therefore representative of a typical cross-section of WWTPs (Table 2). Given the diversity between the WWTPs, microbial community composition and metabolic activity was also expected to vary considerably.

2.2.2. Experimental design

Net ferrocyanide production (i.e. less endogenous control values) was used to determine net FM-respiration for all RAS

Table 2
General characteristics of each WWTP from which activated sludge was used as biocatalyst in this study. RAS and PIS were collected from each WWTP and prepared separately for each FM-BOD assay [1,6]. Design capacity represents maximal hydraulic loading during average dry weather flow conditions.

Plant #	Process	Design capacity ($mL\,d^{-1}$)	Mean sludge age (d)	SSVI ($ml\,g^{-1}$)	[MLSS] ($g\,L^{-1}$)
1	Oxidation ditch	93.2	13.5	78	4.10
2	5-Stage Bardenpho	17[a]	38.3	65	2.86
3	Oxidation ditch	15[b]	26.1	93	7.52
4	Oxidation ditch/mUCT[c]	57.5	12.9	107	4.07
5	5-Stage Bardenpho	7.5	15.6	86	5.32
6	Westbank	1.4	15.0	130	5.05
7	Oxidation ditch	7.5	23.2	103	3.56
8	Oxidation ditch	7.8	32.9	131	3.85
9	Primary sed + MLE[d]	28	15.0	98	3.93
10	Oxidation ditch	66[b]	19.4	107	5.19
11	No data available				

[a] Underloaded – receiving ~30% of capacity.
[b] Overloaded beyond capacity.
[c] Modified University of Cape Town.
[d] Modified Ludzack–Ettinger.