



# Microbial analysis

## Recycled water and biosolids sampling

### Introduction

Recycled water and biosolids (sewage sludge) contain many microbial contaminants that can affect human health. Treated sewage can be treated and the recycled water used to irrigate open spaces such as lawns and gardens, in agricultural and for dust suppression, toilet flushing and other beneficial purposes. Similarly, sewage sludge can be treated to generate biosolids to be used as a fertilizer in agriculture and blended in composts.

In order to ensure the protection of public health, both recycled water and biosolids must be treated to a level that is fit for the intended end-use. A continual sampling program is required to prove that treatment is effective and compliant with either the:

- Guidelines for the Non-potable Uses of Recycled Water in Western Australia (2011); or the
- Draft Western Australia Guidelines for Biosolids Management (2010).

This factsheet provides information on:

- what is recycled water and a biosolid;
- the microbiological test to use,
- why are microorganisms used to assure the safety of the end product,
- the sampling procedures to follow,
- the laboratories accredited to perform the analysis; and
- the analytical methods required by the Department of Health (DoH).

### What are recycled waters and biosolids?

Recycled water is water generated from sewage or from industrial sources that is treated to provide water that is 'fit-for-purpose', for its intended beneficial use. Using recycled wastewater that is 'fit-for-purpose', means it can be safely used for non-potable uses such as open space irrigation, agricultural uses, toilet flushing and a variety of industrial purposes.

Sewage includes:

- Greywater - generated from washing machines, showers, baths, wash basins, spa baths, laundry tubs and when appropriately treated from the kitchen;
- Yellow water is water generated from urinals, and
- Blackwater is water generated from toilets.

Biosolids is sewage sludge generated from a wastewater treatment plant. A biosolid is not dewatered sewage. Biosolids are treated to produce stabilised organic solid residue that have different quality ratings designed specifically to comply with fit-for-purpose quality criteria for



their safe end use. Biosolids may be applied to land as a fertiliser substitute on selected agricultural areas, forestry applications and the production of commercial composts.

## Why test for microorganisms?

Indicator microorganisms are used as a simple and reliable measure of the potential risk to human health.

- Before recycled water can be approved for reuse, you will be required to demonstrate that the water quality after treatment complies with the microbial quality standard that corresponds to the end use risk exposure level.
- After an approval to recycle water has been issued, you will be required to submit microbiological samples periodically to verify that the treatment system continually achieves the required water quality standards.
- Biosolids can be approved for use once compliance with the pathogen grade for the intended end-use has been demonstrated.
- After approval to use biosolids, you will be required to submit microbiological samples to verify that the treatment system complies with the required quality standards.

## What microbial testing do I need to perform?

The type and frequency of microbial analysis will be specified in the conditions of approval provided to you by the DOH. The DOH will set the sample location and frequency to be followed during the validation process and for ongoing verification monitoring. The normal monitoring requirements are as follows:

### Recycled Water

- *E. coli* (an indicator for bacteria) monitoring is required for all risk exposure levels.
- Coliphage (an indicator for viruses) monitoring is required for 'High Risk', exposure schemes.
- Clostridia (an indicator for protozoan oocysts) monitoring may also be required depending on the risk assessment provided to the DOH; and
- Helminths (worms classified as disease-causing organisms) monitoring is required in endemic areas located north of the 20th parallel (mainly the Kimberly region), or where recycled water is used to irrigate pasture and fodder for beef cattle or dairy animals.

### Biosolids

- *E. coli* and *Salmonellae* are commonly monitored and results are reported in counts per gram of dry product.
- Coliphage monitoring may also be required before approval to use biosolids depending on the risk assessment provided to the DoH
- Helminth monitoring may be required before approval where biosolids are to be produced/used above the 20th parallel.



## Why is E. coli testing required?

In the past, total coliform bacteria were used as an indicator of faecal contamination. However, not all coliform bacteria are of faecal origin. Escherichia coli (E. coli) is an indicator of a number of human pathogens that may be present in faeces. Therefore, E. coli is the preferred bacterial indicator organism to test wastewater, recycled water, sewage sludge and biosolids for faecal contamination.

## Why is Coliphage testing required?

Coliphages are viruses that infect coliform bacteria and are considered to be representative of faecally derived viruses. Coliphages have been identified as one of the most tolerant to temperature stress over other bacterial and viral indicators and are useful indicator organisms to evaluate the effectiveness of treatment technologies to remove potential human pathogenic viruses.

## Is a NATA accredited laboratory required?

The DoH requires all microbiological analyses to be conducted by a National Association of Testing Authorities, Australia (NATA) accredited laboratory. NATA Laboratories are formally recognised as technically skilled to perform specific types of testing. To maintain accreditation, the laboratories are re-assessed regularly. NATA accredited laboratories follow standard procedures, methods, inspection, testing and calibration to produce accurate results. The NATA-logo is included in the report of the microbial results.

## How much the testing will cost?

The cost varies between different laboratories and over time. Please contact the NATA accredited laboratories listed below to obtain a quote.

Laboratory	Contact Details
PathWest	J Block, Hospital Avenue QEII Medical Centre NEDLANDS, WA, 6009 Telephone: (08) 9346 3000 Email: <a href="mailto:marketing@parhwest.com.au">marketing@parhwest.com.au</a> <a href="http://www.pathwest.com.au/">http://www.pathwest.com.au/</a>
ProMicro Pty Ltd	31 Green Road HILLARYS WA 6025 Telephone: (08) 9401 5699 Email: <a href="mailto:IT@promicro.com.au">IT@promicro.com.au</a> <a href="http://promicro.com.au/">http://promicro.com.au/</a>
Silliker Microtech Pty Ltd	181 Claisebrook Road PERTH WA 6000 Telephone: (08) 9227 6499 Email: <a href="mailto:sales@silliker.com.au">sales@silliker.com.au</a> <a href="http://www.silliker.com.au/html/silliker/index.php">http://www.silliker.com.au/html/silliker/index.php</a>
MPL Laboratories	16-18 Hayden Court MYAREE WA 6154 Telephone: (08) 9317 2505 Email: <a href="mailto:lab@mpl.com.au">lab@mpl.com.au</a> <a href="http://www.mpl.com.au/">http://www.mpl.com.au/</a>



Laboratory	Contact Details
SGS Australia	10 Reid Road, Perth International Airport, Welshpool WA 6105 Telephone (08) 9373 3556 or 1300 781 744 <a href="http://www.au.sgs.com">http://www.au.sgs.com</a>

## What do I need to do before taking a sample?

Before you take a sample call the laboratory to obtain sample bottles and information on the correct sampling procedure.

For recycled water sampling, read the Recycled Water Sampling Technique Factsheet (<http://www.public.health.wa.gov.au/cproot/2988/2/Recycled%20Water%20Sampling%20Technique.pdf>) and follow all the safety procedures and general rules of sampling.

For biosolids sampling, a number of grab samples will be required. Before you take a sample read the biosolids sampling procedures of the Western Australia Guidelines for biosolids management ([http://www.public.health.wa.gov.au/cproot/1335/2/WA\\_Guidelines\\_Biosolids.pdf](http://www.public.health.wa.gov.au/cproot/1335/2/WA_Guidelines_Biosolids.pdf)).

Please note that your sample will not provide you with accurate information unless the:

- Correct sample bottles are used;
- Correct volume is taken;
- Sample is stored at the required temperature;
- Sample is transported to and arrives at the laboratory within the correct time period; and
- Correct procedure is used to take the sample.

## Analytical Methods Required by the Department of Health

Table No 1 lists the microbial tests for raw or treated recycled water samples and Table No 2 lists the microbial tests for biosolids or composting products.

**Table 1 Recycled Water**

Test	AS Method or validated equivalent	Method	Units/Report
Escherichia coli	AS 4276.6	MPN	MPN/100 mL
Escherichia coli	AS 4276.7	Membrane filtration	cfu/ 100 mL
Escherichia coli	AS 4276.21	MPN using enzyme hydrolysable substrates	MPN/100 mL
Legionella	AS 3896	Direct and selective culture	<10 Legionella Spp./1 mL Count <i>Legionella spp.</i> /mL Count of <i>L. pneumophila</i> including serogroup(s)/mL



<b>Test</b>	<b>AS Method or validated equivalent</b>	<b>Method</b>	<b>Units/Report</b>
Helminths	WHO	Concentration and microscopy	Larvae and Ova / 100mL
*Salmonellae	AS 4276.14	Selective Enrichment	Detected/ Not Detected in the sample volume tested
*Campylobacter	AS 4276.19	Membrane filtration	Presence/absence in the sample volume tested
Clostridium	AS 4276.17.1	Membrane filtration	cfu /100 mL
Clostridium	AS 4276.17.2	Multiple tube dilution technique	MPN/ 100 mL
Enterococci	AS 4276.9	Membrane filtration	cfu/ 100mL
Enterococci	IDEXX	MPN using enzyme hydrolysable substrates	MPN/100 mL
Somatic Coliphage	APHA	Direct culture	pfu/ 100 mL
Somatic Coliphage	APHA. 2005 9224E	single-agar-layer	pfu/ 100 mL
MS2 Coliphage	APHA. 2005 9224E	single-agar-layer	pfu/ 100 mL

\* 1 Litre of sample is recommended

**Table 2 Biosolids and Composting Products**

<b>Test</b>	<b>AS Method or validated equivalent</b>	<b>Method</b>	<b>Units</b>
Escherichia coli	AS 4276.6	MPN	MPN/ 1g (dry weight)
Escherichia coli	AS 4276.7	Membrane filtration	cfu/ 1g (dry weight)
Escherichia coli	AS 4276.21	MPN using enzyme hydrolysable substrates	MPN/ 1g (dry weight)
Legionella	AS 3896	Direct and selective culture	<10 Legionella Spp./1g (dry weight) <b>or</b> Count Legionella spp./ 1g (dry weight) <b>or</b> Count of L. pneumophila including serogroup(s)/ 1g (dry weight)
Helminths	WHO	Concentration and microscopy	Larvae and Ova / 10 g (dry weight)



Test	AS Method or validated equivalent	Method	Units
Salmonellae	AS 4276.14	Selective Enrichment	*Detected/ Not Detected in the sample volume tested
Somatic Coliphage	APHA. 2005 9224	single-agar-layer	pfu/ g
MS2 Coliphage	APHA. 2005 9224	single-agar-layer	pfu/ g

\*Western Australian guidelines for biosolids management require Salmonella report as counts per 50g of dry product

Legionella monitoring may be depending on the risk assessment provided to the DoH

MPN: Most probable number

cfu: colony forming units

pfu: plaque forming units

## Weblinks

Guidelines for the Non-potable Uses of Recycled Water in Western Australia

[http://www.public.health.wa.gov.au/3/1275/2/recycled\\_water\\_guidelines\\_and\\_publications.pm](http://www.public.health.wa.gov.au/3/1275/2/recycled_water_guidelines_and_publications.pm)

Draft Western Australia Guidelines for Biosolids Management

<http://www.public.health.wa.gov.au/3/664/2/biosolids.pm>

Recycled Water Sampling Technique Factsheet

<http://www.public.health.wa.gov.au/cproot/2988/2/Recycled%20Water%20Sampling%20Technique.pdf>

## More information

Water Unit

Environmental Health Directorate

Department of Health

PO Box 8172

PERTH BUSINESS CENTRE WA 6849

Telephone: (08) 9388 4999

Fax: (08) 9388 4910

Produced by Environmental Health Directorate  
© Department of Health, Western Australia 2012

