Biological control tools for wastewater reclamation and reuse. A critical review

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Abstract The health related risk of wastewater reclamation and reuse is usually defined by laws, rules or regulations by using only biological tools; i.e. bacteria, viruses, or other pathogens or indicators determination. Those determinations exert some influence in the costs of the mentioned practices, and it seems probable that in the near future more determinations will be required. Nevertheless, a total indication of wastewater biological quality is not given by such organisms; in fact, long-term toxicity is not detected by such means. The future of biological control of reclamation and reuse systems and the price associated to such determinations is examined through a practical application case.

Keywords Analytical cost; biological control; health hazards; indicator organisms; wastewater reuse

Introduction

All around the world, waterborne bacterial diseases have become far less prevalent, partly because of the intensive research to identify and treat pathogens in drinking water. Nevertheless, fewer efforts have been spent on analysing and treating pathogens in wastewater (Mahin and Pancorbo, 1999). When considering the biological quality of wastewater (raw, secondarily treated and reclaimed), pathogens determination has been quite exclusively limited to bacterial indicators detection and counting, specially those related to *Escherichia coli*, although other organisms are related to health hazards (Yates and Gerba, 1998): viruses, parasites, fungi and algal toxins.

The increase of health hazards and concerns in relation with wastewater, the growing number of wastewater treatment plants, and the need of obtaining additional water resources through wastewater reclamation and reuse, forced the necessity of using more precise and sophisticated biological control tools. It should be considered (Mahin and Pancorbo, 1999) that because of the cost and complexity of analysing actual microbial pathogens, wastewater professionals and regulators have relied for years on traditional faecal indicators (see Table 1) to predict potentially high pathogen levels. Unfortunately, most microbial pathogens are more resistant to conventional wastewater treatment (including chlorination) than pathogen indicators are.

Nowadays, detection and quantification of *E. coli*, the most traditional indicator, are not enough to define the quality of a certain wastewater because they:

- reflect only the bacterial quality and not the presence of other pathogens
- are not good tools to reflect the quality changes due to the use of wastewater treatment processes, conventional or advanced, extensive or intensive
- do not permit the control of reclaimed wastewater disinfection.

Additionally, the long period of time needed to offer results is a negative feature in the analytical work, but it is not only related to *E. coli* determination; quite all biological parameters show the same characteristic. Considering that the presence of a great number

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of pathogens in the wastewater treated, reclaimed, or disposed of in the environment, cannot be stated in terms of *E. coli* presence or quantity, it is necessary to define, if possible, the suitable indicators (see Table 1) in order to establish the biological quality of the different types of wastewater.

Campos (1999) states also the difficulty of defining the quality of the media that receive reclaimed wastewater, particularly the agricultural systems, where reclaimed water is used for irrigation. Plants, soils, groundwater, runoff and atmosphere can and must be controlled separately from the reclaimed wastewater. Campos also indicates the need to establish and realise joint studies of all media implied in a reclamation and reuse system. An additional difficulty is the lack of reference figures for pathogen organisms contents in other media than water.

Otherwise, the current analysis using bacterial indicators detects only the quality of a wastewater single sample, and not the constant quality during a given time, i.e. long-term toxicity. Although toxicity is not directly related to biological quality of wastewater (in all forms) it should be considered because toxicity is determined with bioassays (Olivieri and Eisenberg, 1998).

When critically reviewing (Salgot *et al.*, 1999) laws, rules and regulations on wastewater reclamation and reuse, sampling programmes in order to define the reliability of reclamation technologies are stated in few occasions. Two comments are to be made in this case:

a) not all the reclamation technologies are equally reliable
b) not all the wastewater treatment plants (until secondary treatment) are treating the same amounts of wastewater all around the year; consequently, a temporal change could be defined and it exerts a certain influence on reclamation facilities. This is particularly true when related to tourism areas wastewater treatment plants.

Another system implemented, in relation to reuse facilities, is the detection of the points of risk in reclamation and reuse facilities (Olivieri and Eisenberg, 1998). This is the logical evolution of the good reclaimed wastewater reuse practices (Salgot and Pascual, 1996) and is in some way derived from a really common practice in the foodstuff and in other industries.

Table 1 Types of waterborne pathogens and used indicators (modified from Campos, 1999)

Types of waterborne pathogens	Indicators	Observations
Bacteria	*E. coli*, FC, TC, *Streptococcus faecalis, Staphylococcus, Salmonella, Clostridium*	FC determination is the more usual; *E. coli* determination is slowly substituting it. Other bacteria are used for bathing waters, groundwater, . . .
Viruses	Enterovirus Hepatitis virus Bacteriophage	An accepted indicator still does not exist
Helminths – Nematode	Nematode eggs (*Ascaris*, *Trichuris*, *Ancylostoma* as indicated by the WHO)	Discouraging: a lot of negative results in a lot of countries
Other helminths (i.e. *Taenia*)	Not known	In some cases important for risk related to animal health
Protozoa (include *Giardia, Cryptosporidium, Amoeba*, *Balantidium*, . . .)	Not known The presence of one of them could indicate the presence of the other	Analytical tools not well developed until now
Fungi, algal toxins	Not known	Really few cases detected

Indicator organisms and directly determined organisms

As stated before, several groups of organisms are "determined" by using indicators (i.e. pathogen bacteria and *E. coli*), although others (i.e. *Giardia lamblia*) do not have useful indicators and must be determined directly (see Table 2).

Other aspects

Until now, indicator's based standards have been used to define a suitable reclaimed water quality. Nevertheless, it must be stated that the health risk related to reuse is defined by several aspects: the microbial agent, the human host, and the environment in which the infection process is mediated (Cooper and Olivieri, 1998). As it is the interaction of these components which produces human disease, risk is dependent on defining the exposed population, the microbial characteristics, and the environmental setting in which the exposure occurs. After several considerations, the human health risks associated with the ingestion of waterborne pathogens could be theoretically and numerically defined by using static models that calculate the probability of individual infection or disease as a result of a single exposure event. This type of calculations (Haas, 1983; Regli *et al.*, 1991) could be a good tool when fully developed in the future. A summary can be found in Cooper and Olivieri (1998).

Otherwise, it is suggested in a WHO report (1989), that the epidemiological method must be employed for determining health risks associated with reuse practices; nevertheless, it seems not to be a good tool, as stated by Cooper and Olivieri (1998). It appears that traditional epidemiological methods are not sensitive enough to "tease out" cases that might be associated with recycled water from the background incidence of these ailments in the community.

Up to now, the only system used "legally" for risk control is the definition of water quality according to issued standards.

Economic implications of the reclamation and reuse microbiology

When water is considered a normal component of the socio-economic development, several characteristics or constraints must be identified:

a) the existence of a water market (i.e. a price for any use of the water)
b) the cost of wastewater treatment
c) the cost of wastewater regeneration
d) the cost of reuse
e) the costs associated to controls needed for regeneration and reuse.

Table 2 Organisms usually determined in wastewater treatment, reclamation, and reuse

Type/Organism	Usually/Theoretically employed as	On research	Observations
Total coliforms	Bacterial indicator		Not widely used
Fecal coliforms/*E. coli*	Bacterial indicator	Faster methods	The most used method, despite the problems and discussion
Bacteriophage	Viruses indicator	Most suitable type	Somatic, F specific and *Bacteroides fragilis* HSP40 and RYC2056 phages
Nematode eggs	Nematode and helminth indicator	Better concentration methods. Viability	Recovery of not more than 70%
Giardia lamblia	Direct detection of cysts	Better concentration and detection methods. Viability	In wastewater, false positives can be found in high numbers
Cryptosporidium parvum	Direct detection of oocysts	Better concentration and detection methods.	Viability In wastewater, false positives can be found in high numbers

We are only interested in the development of e). Nevertheless, for more information on the first four, Vergés (1998), Coch and Ludevid (1999), or Caballer and Guadalajara (1998) could be consulted.

Controls on wastewater reclamation and reuse could be analytical or not analytical (good practices, risk definition, . . .) and have several costs associated derived from surveys, sampling or analytical work, either physico-chemical or biological, and analysis interpretation and evaluation. We will only deal in this paper with the definition of costs related to biological analysis.

As stated in almost any regulation, the most usual biological controls are the fecal coliforms, followed by total coliforms and nematode eggs determinations. Viruses, determining either a single species or indirectly bacteriophages, or protozoa are seldom determined by law (Salgot, Pascual and Torrents, 1999). Apart from the setting of such parameters, there are other ways to determine the biological (and sometimes chemical) quality of reclaimed wastewater or to control either the treatment systems or the media where wastewater is reused. We refer to ecotoxicological methods – such as *Daphnia* or Microtox® analysis – or to bioassays, for example: Ames assay, Micronucleus test, 6-Thioguanine resistance assay, and Cellular transformation assay (Cooper and Olivieri, 1998).

Quite every analysis has a different range of prices, as shown in Table 3. When calculating the prices, two considerations must be done:

a) research laboratories usually can operate at lower prices because usually they are heavily subsidised
b) when determining series of samples the price can be reduced.

The capability to perform biological analysis does not guarantee by itself the quality of water or any other media analysed. This work must be undertook under careful planning with respect to the number of samples and the periodicity of sampling.

Obviously, the amount of water used, the risk and the media associated to the reuse, and the extension of the area where wastewater is reused, are influencing the planning of sampling, too.

Case calculation

It is extremely difficult to make calculations for any reuse possibility, so, we limit the calculation only to a single case: agricultural wastewater reuse – unrestricted, one of the most

Table 3 Range of prices in Spain for the biological determinations, in euros (approx. 1 euro = US$ 1.04) as per June 1999

Analysis	Range of prices per a single sample/more than 10 samples	Observations
Total coliforms (TC)	5.0–10.0/4.0–10.0	Not much used in the EU
Fecal coliforms (FC)/*E. coli*	6.0–10.0/5.0–10.0	Classic method
Salmonella	10.0–12.0/9.0–11.0	For aerosol control
Bacteriophage (F+, somatic)	18.0–25.0/15.0–22.5	Under development
Phage of *Bacteroides fragilis*	25.0–35.0/21.0–33.3	Under development
Enteroviruses	150–200/140–175	
Giardia lamblia	242–305/210–275 (*400–500)	Analytical method under development, * if determined together with *Cryptosporidium*, this is the combined price
Cryptosporidium parvum	242–305/210–275 (*400–500)	Analytical method under development, * if determined together with *Giardia*, this is the combined price
Nematode eggs	72–91/61–85	
Daphnia magna test	135–160/130–150	
Bioindicators	50–300/45–280	Depends on the test
Microtox® test	70–85/65–80	

usual ways to reuse regenerated wastewater. The additional characteristics are: conventional, non-seasonal wastewater treatment plant, short residence time, activated sludge, and as a regeneration procedure an infiltration-percolation system (as described by Brissaud *et al*., 1997). We choose the sample pattern described in table 4, based partially on the Catalonia Health Authorities recommendations (Salgot *et al*., 1994; summarised English version Salgot and Pascual, 1996). Soil, plant and groundwater controls, and a few amount of aerosol controls are included. This is a pattern for research purposes; real control could be more reduced (at least half of the optional controls).

When considering the figures obtained from the previous tables, we can evaluate the costs on a range among 5,000 and 90,000 euros (see Tables 4 to 6). Usually, the authorities ask only for the control to be performed on the point of production of regenerated wastewater. Nevertheless, a logical risk control should include a sampling pattern for the affected media, as indicated before.

Table 4 Suggested (research) sampling pattern for a:
(1) conventional, non-seasonal wastewater treatment plant, short residence time, activated sludge and as a regeneration procedure infiltration-percolation plus UV disinfection;
(2) extensive, non-seasonal wastewater treatment plant, long residence time, lagooning
Reuse for agriculture: maximum risk crop

Analysis	No of samples in the first year of reclamation		Parameters to be determined	
	a	b	c	d
Wastewater treatment plant effluent (secondary)	156 26 13	52 26 13		FC, Bacteriophage, Nematode eggs. *Giardia* & *Cryptosporidium*
Pretreatment (tertiary without disinfection)	52 26 26	52 13 13		FC, Bacteriophage, Nematode eggs. *Giardia* & *Cryptosporidium*
Tertiary effluent (including disinfection).	64[e]	19[e]	FC, Nematode eggs	
a) Wastewater treatment plant effluent (including disinfection)	156 26 13	52 13 13		FC, Bacteriophage, Nematode eggs. *Giardia* &
b) Lagooning	6	6		*Cryptosporidium* Toxicity, bioassays
Water in the point of use (seasonal)	26 for all parameters			FC, Bacteriophage, Nematode eggs. *Giardia* & *Cryptosporidium*
Vegetation (seasonal)	26 for all parameters			FC, Bacteriophage, Nematode eggs. *Giardia* & *Cryptosporidium*
Soil (seasonal)	26 for all parameters			FC, Bacteriophage, Nematode eggs. *Giardia* & *Cryptosporidium*
Groundwater	13 6			FC, Bacteriophage Toxicity, bioassays
Aerosols	13 for all parameters			FC, Bacteriophage, *Salmonella*[f]

a: wastewater treatment plants with water retention time > 24h
b: wastewater treatment plants with water retention time < 24h
c: indicated by the recommendations
d: suggested for full characterisation of risk
e: the recommendation only indicates reclaimed effluent control
f: *Salmonella* appears to survive better in aerosols than FC (Crook, 1998)

Table 5 Analytical costs according to the pattern defined by previous table

Analysis	Unit price per more than 10 determinations (see Table 3)	Number of analysis/Total price	
		a	b
FC	7.5	532/3990	279/2092
Bacteriophage 1	8.7	468/8752	260/4862
Nematode eggs	73	220/16060	149/10877
Giardia & *Cryptosporidium*	450	130/58500	117/52650
Microtox® test	72.5	12/870	12/870
Daphnia magna test	140	12/1680	12/1680
Salmonella	10	13/130	13/130
TOTAL		1387/89982	842/73161

Table 6 Costs not related to analysis

Item	Cost includes	Cost	Observations
Survey	Supervisor time	2,200 euros/year	4 surveys· year^{-1} Inspection cost can or cannot be included in the budget of Administration
Interpretation	Researchers time	600 euros/year	60 euro/hour.
Editing		190 euros/year	
Sampling and transporting	Per diem and transportation media	600 euros/year	Estimating an average 100 km
TOTAL		3,590 euros/year	

Discussion

It seems obvious that the analytical controls usually recommended for reuse facilities are not enough to guarantee either a lack of risks or an acceptable level of risk. Usually, controls are limited to reclaimed wastewater at the point of production of such water. Nobody seems to be capable of issuing rules and regulations on the biological quality of : (a) the point of use of water; (b) the quality of receiving media (surface waters, vegetables, soil, air, groundwater).

The control is quite exclusively limited to the risk generated by bacteria, which does not correspond to the global health hazard, where viruses, nematodes and protozoa are also implied. Reliability and variability of reclaimed water is also forgotten when reclamation and reuse facilities are considered. Another lacking tool is the capability to detect the disinfection effectivity of the wastewater and reclamation equipment.

Nevertheless, the introduction of such control measures will have a certain impact: (a) on the amount of control tasks needed; (b) on the price of reclaimed wastewater.

Conclusions

From the health point of view it seems necessary to define the control mechanisms on the different parts of a reclamation and reuse systems: wastewater treatment, reclamation facility, media where reclaimed wastewater is applied directly or indirectly.

From the economic point of view, the biological analytical work for a wastewater reclamation system increases the costs of the reuse in a range between 0.10 and 0.030 euros/m^3.

If the risk is defined only with the use of bacterial indicators (*E. coli*) and nematode eggs, the costs are relatively reduced and correspond to the lower figures. Nevertheless, in such a case, the risk level is not well defined.

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