Comments on: *Direct extractions of proteins to monitor an activated sludge system on a weekly basis for 34 weeks using SDS-PAGE* by MM Ehlers and TE Cloete (1999) (*Water SA* 25 (1) 57-62)

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1. Choice of SDS-PAGE method

Ehlers and Cloete evaluated the SDS-PAGE test method, as applied to activated sludges, and have reported the results elsewhere (Ehlers et al., 1998 p. 51). One set of these results (1998) showed that the SDS-PAGE method was not able to distinguish between three different sludge samples. The first sample contained pure *Acinetobacter calcoaceticus* ATCC 23055, the second sample contained a mixture of activated sludge and *Acinetobacter calcoaceticus* ATCC 23055 in a ratio of 2:1, and a third sample contained a 1:1 mixture of the same cultures that had been allowed to stand for 24 h. The percentage correlation of these three mixtures with activated sludge were all reported as 59%.

Ehlers and Cloete (1999) state that one of their aims was to determine whether there were any differences between the sludges in the anaerobic, anoxic and aerobic zones of a three-stage Bardenpho activated sludge plant. The results described in the previous paragraph indicate that the SDS-PAGE technique is not the appropriate technique to detect such differences, as this plant configuration (a three-stage Phoredox) belongs to the *single-sludge,* multiple-reactor group of processes (Ekama et al., 1984). The system sludge is recirculated to the start of the reactor sequence and should therefore only experience relatively gradual composition changes.

2. Reported phosphate levels

The Daspoort Treatment Works must comply with the special phosphate standard for effluent discharge, yet the results reported by Ehlers and Cloete (1999) indicate that the aerobic reactor of the plant has phosphate levels above the 1 mg/ℓ discharge limit. This might mean that the aerobic sample was collected from a point near the start of the aerobic reactor series, in which case the sludge protein profile will also not be representative of aerobic sludge.

The phosphate concentration of the treated water (after clarification) is monitored continuously at the Daspoort Treatment Works and an average value is recorded every hour. The average daily value for the works is listed in the following table ("Works") to allow comparison between these plant values and those reported by Ehlers and Cloete ("Report"). The phosphate level detected by the Daspoort monitoring system can be as low as that in the last aerobic reactors, but is sometimes slightly higher, due to small amounts of phosphate being released in the clarifier sludge blanket.

Sample	Phosphate mg/ℓ		Sample	Phosphate mg/ℓ		Sample	Phosphate mg/ℓ	
Date	Report	Works	Date	Report	Works	Date	Report	Works
2/4	3.86	0.34	1/7	7.06	0.19	23/9	3.1	0.18
16/4	0.73	0.95	11/7	0.44	0.47	30/9	6.69	0.14
22/4	3.44	0.98	15/7	7.64	0.16	7/10	2.28	NR
29/4	6.8	1.52	22/7	1.9	0.21	14/10	0.32	NR
7/5	7.14	0.45	29/7	5.64	0.14	21/10	3	NR
14/5	0.62	0.20	5/8	0.45	0.11	28/10	7	NR
20/5	7.78	0.25	12/8	1.66	0.62	4/11	0.34	0.02
27/5	0.67	0.21	19/8	4.57	0.23	11/11	0.58	0.04
3/6	8.99	0.18	26/8	1.2	0.91	18/11	0.87	0.04
10/6	0.56	0.18	2/9	2.45	0.38	25/11	0.46	1.99
18/6	7.23	0.08	9/9	3.34	0.31			
24/6	0.59	0.11	16/9	5.01	0.26			

NR = Not recorded

There is no apparent correlation between the aerobic reactor phosphate levels reported by Ehlers and Cloete (1999) and that recorded by the Daspoort monitoring system. The change in phosphate levels (delta P) across the plant reported by Ehlers and Cloete (1999) therefore probably does not reflect the actual phosphate removal and should not be used to evaluate any correlation between the phosphate removal and the SDS-PAGE profile.

The anaerobic reactor phosphate levels reported by Ehlers and Cloete (1999) are also much lower than the anaerobic reactor levels normally recorded in the Daspoort plant (Saayman, 1999).

Conclusion

Ehlers and Cloete (1999) appear to have applied an inappropriate analytical method to a single sludge system, and have investigated the correlation between these results and incorrect phosphate readings.

References

EHLERS MM, ERASMUS A and CLOETE TE (1998) Fingerprinting of Activated Sludge Systems Using Page Analysis of Total Protein Extractions For the Optimization of Biological Phosphorus Removal. Water Research Commission Report No. 776/1/98. Pretoria, South Africa.

EKAMA GA, MARAIS GvR, SIEBRITZ IP, PITMAN AR, KEAY GFP, BUCHAN L, GERBER A and SMOLLEN M (1984) Theory, Design and Operation of Nutrient Removal Activated Sludge Processes. Water Research Commission, Pretoria, South Africa.

SAAYMAN G (1999) Personal communication. Pretoria.

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Authors' reply

1. **Choice of SDS-PAGE method:** Mr Smith refers to another publication (WRC Report No. 776/1/98) which has been discussed with him. This publication indicated the sensitivity of the SDS-PAGE method where mixtures of sludge and a pure *Acinetobacter* culture were compared. The percentage of similarity between the samples was clearly indicated by the dendrogram and not the table he referred to.
2. Mr Smith suggests that the microbial composition would not differ in the system studied due to the configuration (single-sludge, multiple-reactor group processes etc.). Our results support this notion indicating the appropriateness of SDS-PAGE since this is exactly what was confirmed.
3. **Reported phosphate levels:** After discussions with Mr Esterhuizen of Daspoort activated sludge plant, we determined that the phosphorus analysis Mr Smith referred to was from the automatic sampler which measures the phosphorus concentrations of the effluent. Our results from Daspoort laboratories were the phosphorus concentrations measured within each zone of the system.
4. Mr Smith suggests that the SDS-PAGE data cannot be correlated with phosphate removal. The idea in our study was not to correlate SDS-PAGE with phosphorus removal, but to study the microbial community structure amongst the different zones of an activated sludge plant over time.

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