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# Influent pathogenic bacteria may go straight into effluent in full scale wastewater treatment plants



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## Introduction

Microorganisms from wastewater are usually considered to be adsorbed onto the activated sludge flocs in wastewater treatment plants (WWTPs), consumed by protozoan or to die off. Therefore, the effluent is assumed to comprise primarily of those microorganisms that grow in the plant.

Standard techniques for detecting bacteria, particularly pathogens, in the effluent are based on culture-dependent methods, which may underestimate potential pathogenic bacteria escaping WWTPs in the effluent.

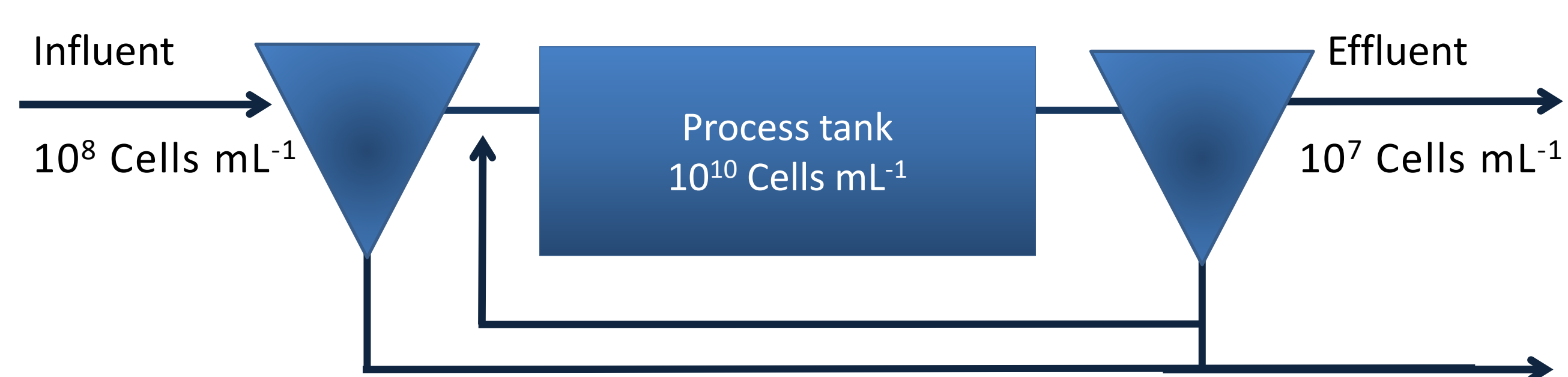
Culture-independent DNA sequencing methods may resolve that.

## Objective

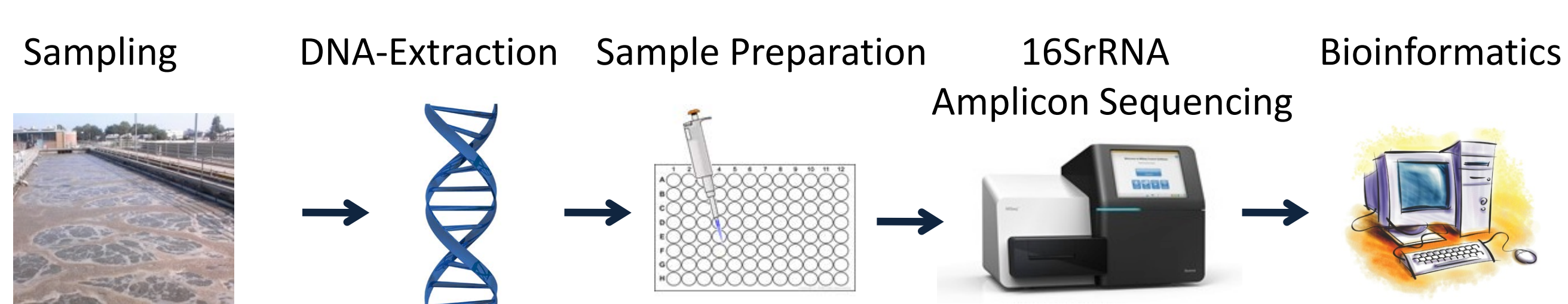
- To determine if microorganisms from the incoming wastewater are being incorporated in the activated sludge community, die off, or whether they stay dispersed in the water phase, and thus are discharged from the WWTPs along with the effluent.

## Methods

Analysis of the microbial composition, combined with bacterial mass balances to calculate net growth and relative abundances in the influent, process tank and effluent of 14 Danish full-scale WWTPs.



The microbial community composition was examined by 16S rRNA gene amplicon sequencing (V1 to V3 region) and MiDAS curated taxonomy (<http://midasfieldguide.org/>).



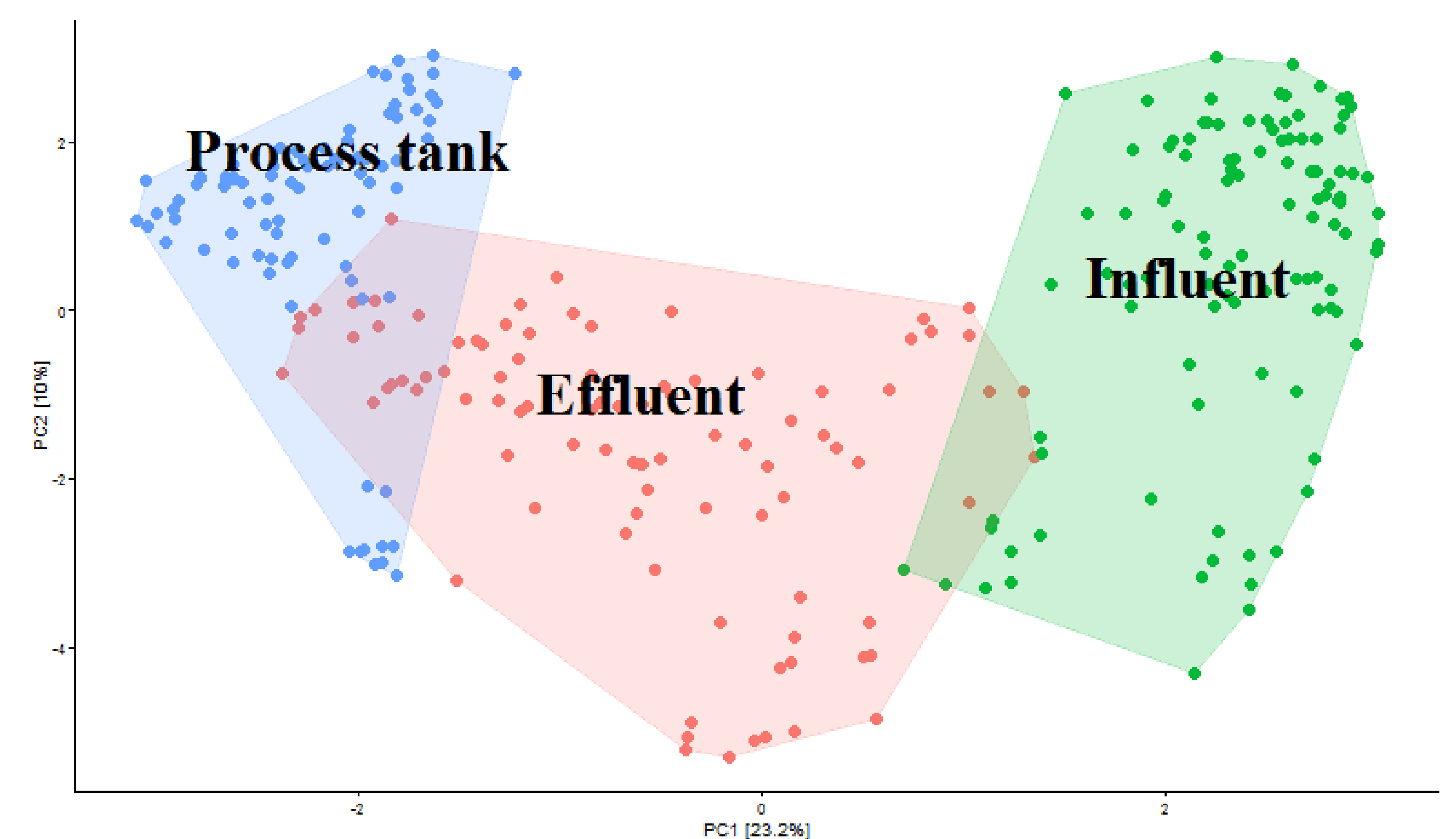
## Conclusions

The effluent microbial community was in some WWTPs very similar to the process tank community, in other plants influent and effluent communities were very similar while in some plants it was a combination.

This indicates that in some plants many microorganisms may go straight through the plant from influent to effluent without being incorporated into activated sludge flocs.

The non-growing genus *Arcobacter* was among others found abundant in effluent. Some *Arcobacter* species are known pathogens, and failure to remove these from wastewater pose a potentially serious health safety problem.

## Results



### Community composition

The figure shows the relationship between all samples analyzed from 14 WWTPs. Samples are grouping after sampling location (Influent, Process tank and Effluent) with a clear difference between Influent (Green) and Process tank (Blue) composition, and Effluent (Red) community composition appearing in between.

	Influent					Process tank					Effluent			
<i>Arcobacter</i>	7	33.3	20.9	23.4	27.7	0.4	0.9	1.2	1	0.9	8.9	12.5	19.7	29.9
<i>Streptococcus</i>	5	7.7	11.9	10.7	8.1	0.8	1	1	0.9	1	2.1	4.8	4.2	4.8
<i>Trichococcus</i>	8.1	2.7	2.5	3.5	4.4	2.5	2.4	3.9	3	3.8	1.1	0.6	0.7	0.7
<i>Cloacibacterium</i>	4.3	4.7	3.2	3	3.4	0.2	0.3	0.2	0.2	0.2	4	4.2	4.8	5.5
<i>Acidovorax</i>	9.3	2	4.1	3.1	2.8	1.7	1.2	0.8	1.3	0.9	1.6	2	1.7	1.3
<i>Blautia</i>	2.5	1.7	2.8	3.2	3.3	0.1	0.2	0.2	0.4	0.4	0.6	0.5	0.7	0.6
<i>Comamonas</i>	2.8	2.5	3.2	2.1	1.3	0.2	0.2	0.3	0.2	0.1	0.4	0.9	1.1	0.8
<i>Faecalibacterium</i>	2.5	2.1	3.3	2.5	2.5	0.1	0.1	0.1	0.1	0.1	0.6	0.6	0.7	0.5
<i>Bacteroides</i>	1.2	2.2	1.3	1.6	1.5	0	0.1	0.1	0.1	0.1	1.3	1.3	1.5	2
<i>Prevotella</i>	1.7	2.5	2	1.9	1.6	0	0.1	0.1	0.1	0.1	0.8	0.9	0.9	0.9
	24-10-2014	09-11-2014	19-11-2014	25-11-2014	09-12-2014	24-10-2014	09-11-2014	19-11-2014	25-11-2014	09-12-2014	24-10-2014	09-11-2014	19-11-2014	25-11-2014

### Relative abundance

The 10 most abundant genera are shown at different time points in influent, process tank and effluent of a Danish WWTP. Calculation of net growth rates and mass balances for all OTUs confirmed that *Arcobacter* did not grow in the plants. This indicates that *Arcobacter* cells found in the effluent originated from the influent wastewater.