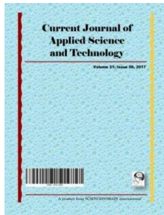

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Modeling for Collecting Kinetic and Microbial Population to Study Bio-P Elimination

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Authors' contributions

This work was carried out in collaboration between both authors. Author AA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SC managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

A SBR (Sequencing Batch Reactor) was used to study the kinetics of the processes involved in the Enhanced Biological Phosphorus Removal (EBPR) from wastewater. In addition to the batchwise operated reactor, two types of continuous plants, an A^2/O (Anaerobic-Anoxic-Oxic) system and a UCT (University of Cape Town) system, have been operated to cultivate phosphorus removing sludge. Growth of phosphorus accumulating organisms has been established. Concentration profiles indicate the possibility for complete phosphorus removal during the aerobic period. Kinetic models have been calibrated for anaerobic phosphate release and aerobic phosphate uptake. The models have been validated using experimental data from the different plants. The bacterial population of both the A^2/O and the SBR remained stable over the period tested. However, the majority of the organisms isolated from the A^2/O belonged to the Enterobacteriaceae, while the population of the SBR system was different. Representatives of Acinetobacter, regarded as a possible causal agent for biological phosphate removal, were only occasionally present in the sludge.

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Keywords: Sequencing batch reactor; kinetics; biological phosphorus removal; aerobic.

SYMBOLS

The following list of symbols is mainly related to the equations:

$C_{PHB,X}$ *Internal PHB content of the biomass*
C_{AC} *Acetate concentration in the mixed liquor*
C_{PO4} *Orthophosphate concentration in the mixed liquor*
f_{pp} *Phosphate release to PHB accumulation ratio*
K_{PHB} *Rate constant for PHB accumulation*
K_{pp} *Rate constant for PHB consumption*
K_{Ac} *Half saturation constant for acetate*
K_{PP} *Half saturation constant for internal polyphosphate*
n *Number of measurements*
y *Observed concentration of considered compound*
v *Number of responses*
$X_{PP,X}$ *Internal polyphosphate content of the biomass*
X_{PAO} *Amount of poly-P bacteria*
Acetate uptake to PHB accumulation ratio

1. INTRODUCTION

For ecological reasons biological phosphorus removal from waste water is preferred to chemical phosphate precipitation [1]. A conventional waste water treatment plant can easily be converted to a biological phosphorus removal plant by providing an anaerobic tank preceding the aerobic reactor. Batch operated processes such as the SBR have the advantage that a stable operation can easily be obtained [2], allowing frequent sampling without disturbing the process. Continuous installations, however, are closer to real field situations. Therefore the SBR is mainly operated to record concentration profiles in time, to be used for kinetic modeling [3].

2. METHODS

2.1 Apparatus

The study was carried out in laboratory ferment or filled with 8L of mixed liquor. The reactor was operated as a sequencing batch with a cycle length of 6 or 8 hours: 15 min filling (anaerobic), 1.5 hours anaerobic phase, 3.5 or 5.5 hours aerobic phase, a maximum of 10 min anoxic in between phase, a minimum of 33 min settlement phase and a 17 min effluent withdrawal period. At the end of the aerobic period, 200 ml of mixed liquor was purged to obtain a sludge age of 10 or 13 days.

An amount of 3.8 litres of supernatant was removed at the end of the cycle and 4 litres of medium was fed at the beginning of the cycle. The hydraulic retention time thus was 12 or 16 hours. A time controller was used to obtain the settings for the different phases [4].

During the anaerobic phase, the mixed liquor was sparged with nitrogen. During the aerobic phase the dissolved oxygen (DO) concentration was controlled in an on-off strategy with a set point of 2 mg O_2/l. In practice values of 2 ± 0.5 mg O_2/l occurred due to overshoots in both directions. Gas flows were controlled with mass flow controllers. Flow rates were fixed at a given value for nitrogen and air and could only be changed manually [5].

The A^2/O and the UCT systems consisted both of an anaerobic reactor, an anoxic reactor, an aerobic reactor and a settler. The main difference between both configurations is that the sludge recycle is directly pumped to the anaerobic zone for the A^2/O unit, and for the UCT unit to the anoxic zone with an extra internal recycle from the anoxic zone to the anaerobic zone. A sludge age of 10 days was aimed at. The reactor volumes of both systems were adjusted to obtain the same residence times. The anaerobic residence time (1h20) and the aerobic residence time (3h50) were comparable to the lengths of these phases in the SBR [6]. The residence times in the anoxic zone and in the settler were much higher for the continuous set-ups. The influent flow rate equaled 0.96/h.

2.2 Medium

For the SBR, in a first experimental phase, a non-sterilized medium was used containing per liter: 0.85 g $NaAc.3H_2O$ (400 mg COD), 65.81 mg KH_2PO_4 (15 mg P), 90 mg $MgSO_4.7H_2O$,14 mg $CaCl_2.2H_2O$, 36 mg KCl, 107 mg NH_4Cl (28 mg N), 1 mg yeast extract, 275.4 mg $NaHCO_3$ and 0.3 ml nutrient solution. This mixture was adopted from [7], adding $NaHCO_3$ as a buffer agent. In the second phase the COD concentration was doubled. In a third experimental phase the composition per liter

was changed to : 1.7 g $NaAc.3H_2O$ (800 mg COD), 197.43 mg KH_2PO_4 (45 mg P), 180 mg $MgSO_4.7H_2O$, 28 mg $CaCl_2.2H_2O$, 72 mg KCl,214 mg NH_4Cl (56 mg N), 2 mg yeast extract, 275.4 mg $NaHCO_3$ and 0.6 ml nutrient solution.

The nutrient solution contained per liter: 1.5 g $FeCl_3.6H_2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4.5H_2O$, 0.18 g KI, 0.12 g $MnCl_2.4H_2O$, 0.06 g $Na_2MoO_4.2H_2O$, 0.12 g $ZnSO_4.7H_2O$, 0.15 g $CoCl_2.6H_2O$ and 10 g EDTA. Only reagent grade products were used [8].

The A^2/O and UCT were fed with a meat extract mixture consisting of 3.125 ml meat extract (Liebox) and 82.26 mg KH_2PO_4 on liter basis the influent thus contained 1450 mg COD/*l* and 29 mg P/*l*.

2.3 Isolation Procedure

The composition of the bacterial population of the A^2/O and SBR experiments has been studied after a direct isolation procedure. Samples were plated after homogenization and serial dilution on eight different media (general as well as specific) for colony forming units (cfu). The most abundant two or three different colony types were selected from each type of medium for further purification [9].

2.4 Analysis Methods

Orthophosphate analyses, by means of the ascorbic acid method, nitrate measurements, by means of the hydrazine reduction method, and ammonia measurements, using the Berthelot reaction, were performed with a colorimetric autoanalyser. Colorimetric COD analyses were performed according to the Standard Methods using prefabricated tubes. Potassium measurements were conducted on a flame photometer. Probably due to interference with the high sodium content, the measured potassium concentration exceeded the actual one [10]. Since the sodium concentration remained constant, the interference was considered constant. For polyhydroxyalkanoate (PHA) analyses, lyophilized biomass was subjected to a propylation reaction. The organic phase was analyzed by gas chromatography. Polyphosphate was calculated from a total phosphate analysis. Therefore, lyophilized biomass was subjected to a destruction reaction in an H_2SO_4-HNO_3-mixture (1:1). Organic phosphates were thereby converted to orthophosphate and measured as such. The polyphosphate content was calculated from this total phosphate content by subtracting the phosphorus content of the biomass (1.8%). For MLSS measurement 25 ml mixed liquor Biological Phosphorus Removal: Composition of microbial population and kinetic modeling was filtered on Whatman glass microfibre filters (GF/C). Before and after filtration, the filters were dried during two hours at 105°C and weighed [11].

2.5 Identification of the Microbial Population

All isolates were characterized by gas chromatographic analysis of their methylated fatty acids (FAME) [7]. The FAME patterns were compared with the Microbial Identification Software (MIS) database (TSBA version 3.9, Microbial ID Ind.; Newark, Delaware, USA). The obtained fatty acid profiles were compared numerically and the strains were grouped in a dendrogram according to the similarities of their fatty acid profiles [12].

The stability of the bacterial population during the three month sampling period was studied by FAME analysis of the sludge. The obtained FAME profiles of the different samples (about 100 mg of centrifuged sludge) were compared numerically and expressed as Euclidean distances. Generally, an Euclidean distance of 13 or less is accepted as within bacterial species variability [13].

2.6 Parameter Estimation

For modeling purposes, samples were taken every five minutes, gradually enlarging the sampling interval to 20 minutes towards the end both anaerobic and aerobic phases when limited changes were expected. The experimental data were initially confronted with basic equations, gradually taking more compounds into account, until adequate description of the experimental profiles was obtained. The parameters in the models were estimated on the basis of the generalized least squares criterion for multi-response systems, using a Levenberg-Marquardt algorithm.

$$\sum_{i=1}^{V}\sum_{j=1}^{V} W_{ij} \sum_{k=1}^{n} (y_{ki} - \hat{y}_{ki}).(y_{kj} - \hat{y}_{kj}) \longrightarrow min$$

3. RESULTS AND DISCUSSION

3.1 Experimental Profiles

In Fig. 1, a typical profile recorded during an experimental run performed on the SBR, is presented. Anaerobically orthophosphate is released, whereas acetate is consumed with concurrent formation of PHA, particularly PHB and PHV. Aerobically, phosphate is stored internally by the bacteria using PHA as internal carbon source. Potassium follows the same profile as orthophosphate, according to the formula $Mg_{1/3}K_{1/3}P$. The feed composition from the third experimental phase was used.

3.2 Modeling the Aerobic Processes

During aerobic conditions PHA, which was stored in the preceding anaerobic phase, is oxidized by the phosphorus accumulating bacteria and orthophosphate is converted to polyphosphate. The performed experiments were designed to allow all soluble carbon sources to be converted during the anaerobic phase. Hence, aerobic growth of heterotrophic bacteria on soluble carbon sources is hindered, while concurrent nitrification, and hence chemolithotrophic growth, can take place [14].

The uptake of phosphorus for the synthesis of the polyphosphate chain is linked to the oxidation of PHA, especially PHB. According to the metabolic model developed by [11] for every mole of phosphate uptake, 0.27 C-mole of PHB is oxidized (5.34 g P/g PHB). The fraction of PHB used in the aerobic phase for biomass synthesis is also taken into account. However, the amount of orthophosphate necessary for this biomass synthesis and the biomass dynamics were neglected. According to several authors [11] PHB is also used for glycogen formation. However, since no glycogen measurements were available, no glycogen dynamics were modeled. Hence the necessary PHB conversion is lumped into the growth term. To account for orthophosphate limitation towards the end of the aerobic period, a switching function was introduced for orthophosphate. The affinity constant was chosen upon 0.01 mg P/l. It was noticed that the uptake of orthophosphate was substantially higher when the polyphosphate content of the biomass was low. To account for this phenomena a Monod-equation, not used in the IAWQ No 2 model was introduced for polyphosphate [15].

The polyphosphate content was measured only at the beginning of the aerobic period. Therefore the polyphosphate content at any time was calculated from the orthophosphate profile, according to the following mass balance:

$$X_{PP,X}(t) = X_{PP,X}(0) + \frac{C_{PO_4^{3-}}(0) - C_{PO_4^{3-}}(t)}{X_{PAO}}$$

In the following the model for polyphosphate uptake and PHB utilization is presented. 91 orthophosphate- P measurement points and 54 PHB measurement points served the purpose of parameter estimation. Initial orthophosphate and PHB concentrations, i.e. at the beginning of the phase, were considered unknown and hence treated as parameters. Tying up the model onto the initial values for each experiment is inconsistent with the fact that they are also are subject to experimental error.

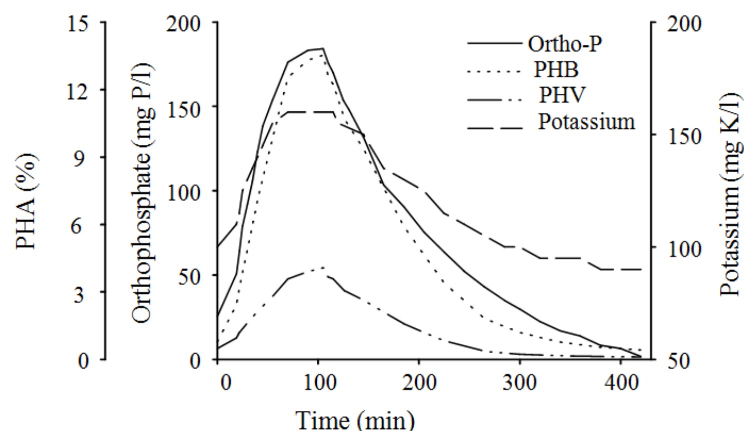

Fig. 1. Typical profile recorded during an experiment

The selected model reads:

$$\frac{dC_{PHB,X}}{dt} = -k_{PP}.\frac{C_{PHB,X}}{K_{PHB} + C_{PHB,X}}.\frac{C_{PO_4^{3-}}}{0.01 + C_{PO_4^{3-}}}.\frac{K_{PP} + X_{PP,X}}{X_{PP,X}} - k_x.C_{PHD,X}$$

$$\frac{dC_{PO_4^{3-}}}{dt} = -5.34.k_{PP}.\frac{C_{PHB,X}}{K_{PHB} + C_{PHB,X}}.\frac{C_{PO_4^{3-}}}{0.01 + C_{PO_4^{3-}}}.\frac{K_{PP} + X_{PP,X}}{X_{PP,X}}.X_{PAO}$$

Table 1 presents the statistical analysis. Estimation of all parameters together resulted in identifiability problems [16]. The value for K_{PP} has thus been estimated separately, and hence no statistical analysis is available for this parameter. As can be seen from the statistics and from the parity plots in Figs. 2 and 3, the agreement between observed and calculated values is very good. Observed and calculated values for one experiment are presented in Figs. 2 and 3.

The model was validated using two experiments performed during a transient stage of the SBR and for batch experiments conducted on sludge taken from the A^2/O and UCT systems. Since in non of these cases it was expected that all biomass consisted of polyphosphate accumulating organisms, the fraction of these organisms was considered as a parameter. The model could also be validated on the experiments performed on the A^2/O and UCT systems, with low initial oxygen concentration, by incorporating an oxygen switching function.

3.3 Modeling the Aerobic Processes

During the anaerobic phase the carbon sources from the feed are converted to PHA. The energy required for acetate uptake is provided by breakdown of polyphosphate formed during the preceding aerobic phase. Nitrate, formed also during the preceding aerobic phase, is used very rapidly as a terminal electron acceptor, so that the nitrate concentration sharply drops to zero already after a few minutes. True anaerobic conditions thus are nearly present throughout the whole "anaerobic period".

Because acetate and lactate feeds were used, mainly PHB and PHV were formed. Acetate is mainly converted into PHB, with a minor fraction into PHV. Lactate is mainly converted to PHV. Although both PHB and PHV show the same anaerobic-aerobic profiles, experiments indicated that particularly PHB is used during the aerobic period for phosphate storage. It is thought that PHV and PHB form a copolymer with varying composition according to the amount of lactate fed. For modeling purposes only acetate feed were considered and model development was done on the basis that acetate is only converted to PHB. Though it is suggested that PHB is also formed from glycogen, stored in the preceding aerobic phase [7], this process has again not been taken into account, because of a lack of glycogen data.

Beside initial orthophosphate and PHB concentrations, also the initial acetate concentrations were considered as parameters, for reasons mentioned above.

The final model selected is:

$$\frac{dC_{PHB,X}}{dt} = k_{PP}.\frac{C_{Ac}}{K_{Ac} + C_{Ac}}.\frac{X_{PP,X}}{K_{PP} + X_{PP,X}}$$

$$\frac{dC_{PO_4^{3-}}}{dt} = f_{pp}k_{PHB}.\frac{C_{Ac}}{K_{Ac} + C_{Ac}}.\frac{X_{PP,X}}{K_{PP} + X_{PP,X}}X_{PAO}$$

$$\frac{dC_{Ac}}{dt} = -\beta.k_{PHB}.\frac{C_{Ac}}{K_{Ac} + C_{Ac}}.\frac{X_{PP,X}}{K_{PP} + X_{PP,X}}X_{PAO}$$

Table 2 presents the statistical analysis. Again estimation of all parameters together resulted in iden- tifiability problems. The value for K_{PP} has thus been estimated separately. Although the agreement between observed and predicted values is in this case somewhat less accurate then for the aerobic processes, the fit again is very good (Figs. 4 and 5). The value for K_{Ac}, however, is less precisely estimated as the other parameters. By way of example observed and calculated values for one experiment are presented in Figs. 6 and 7.

Validation of the selected model using the batch experiments conducted on sludge taken from the A^2/O and UCT systems, was not yet very good. Only low acetate concentrations were used in those experiments. Calibrating the selected model using only the latter experiments, resulted in a first order for acetate and a zero order for the internal polyphosphate content. More experiments, using a broader range of feed concentrations should be performed for accurate validation of the selected model.

Table 1. Parameters and statistical analysis for the aerobic phosphate uptake and PHB utilization 95% confidence interval

Parameter	Optimal value	Lower limit	Upper limit
K_{pp}	5.23	4.77	5.68
K_x	0.67	0.65	0.69
K_{PHB}	50.75	42.34	59.16
K_{pp}	2.0	-	-
Estimated error covariance matrix (PHB, phosphate)		5.97 -1.63	-1.63 5.13

Table 2. Parameters and statistical analysis for the anaerobic phosphate uptake and PHB utilization 95% confidence interval

Parameter	Optimal value	Lower limit	Upper limit
K_{PHB}	250.33	200.74	299.91
K_{AC}	111.77	53.25	170.28
K_{pp}	10.0	-	-
f_{PP}	0.37	0.36	0.39
β	0.92	0.88	0.96
Estimated error covariance matrix (PHB, phosphate)	37.84 37.84 4.32	4.32 4.31 7.89	-35.82 -5.37 1475.6

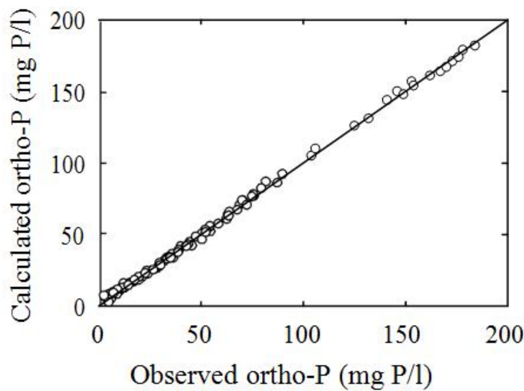

Fig. 2. Parity plot for orthophosphate

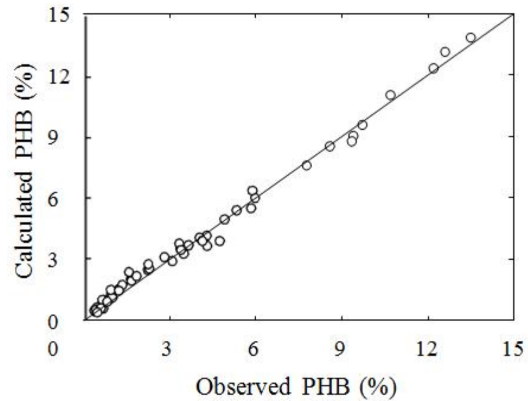

Fig. 3. Parity plot for PHB

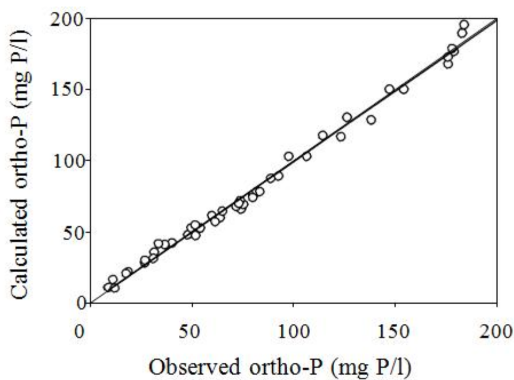

Fig. 4. Parity plot for orthophosphate

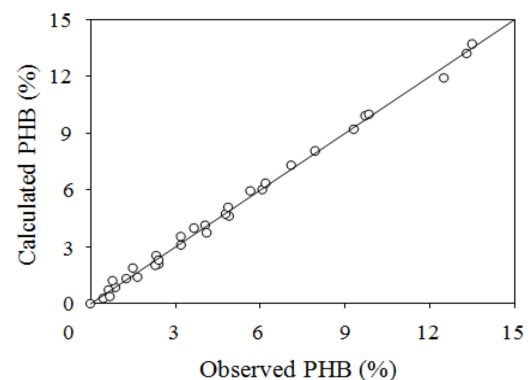

Fig. 5. Parity plot for PHB

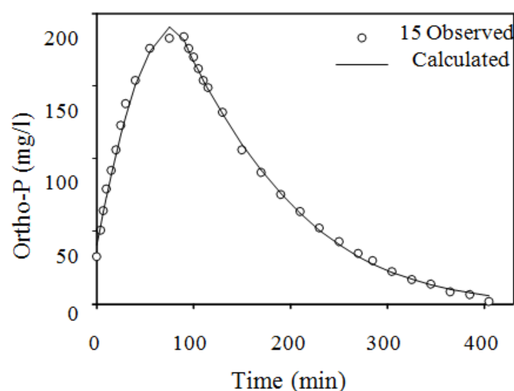

Fig. 6. Observed and calculated orthophosphate profile

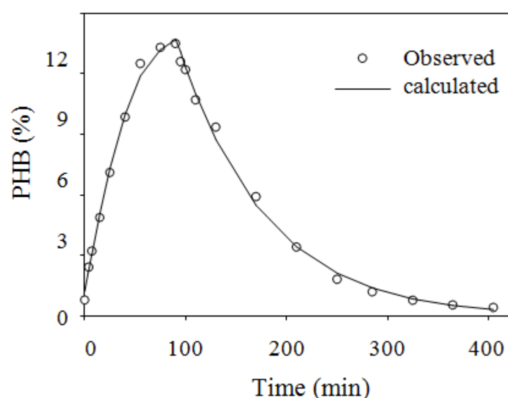

Fig. 7. Observed and calculated PHB profile

3.4 Applicability Range of the Activated Models

The models have been calibrated using feedstock containing acetate ranging between 400 and 800 mg COD/*l* and orthophosphate ranging between 15 and 30 mg P/*l* .The aerobic and anaerobic orthophosphate concentrations ranged between 0 and 200 mg P/*l* and the internal PHB contents between 0 and 14%. The selected models thus are only valid in mentioned concentration ranges.

3.5 Composition of the Microbial Population

More than 60% of the isolates could be identified with the MIS data base at least at the genus level. In the A^2/O installation, representatives of the *Enterobacteriaceae* formed the most dominant group (50% of all isolates). On the contrary, only 2% of the isolates of the SBR were identified as belonging to the *Enterobacteriaceae*. On the contrary, four different groups of organisms were present according to the performed FAME analysis. They represented each about 10% of the total bacterial population: two unidentified groups, a group belonging to the genus *Pseudomonas* and a group containing representatives of the lactic acid bacteria. However, experience has learned that reliable identification of lactic acid bacteria by FAME profiling is doubtful (Pot, personal communication), which means that only part of the SBR population could be identified.

This striking difference of bacterial population of the SBR and the A^2/O experimental set-ups is supported by a different FAME profile exhibited by the direct analysis of both sludge (Euclidean distance >17). During the sampling period the fatty acid composition of the SBR and A^2/O remained fairly stable (within Euclidean distance lower than 10) which supports the stability of the system during this period. The striking absence of *Acinetobacter* species (only one strain was isolated) confirmed again [17] that this organism is not a prerequisite for biological phosphorus removal.

Former diaminopropane analysis (DAP) of the sludge supports this observation.

4. CONCLUSION

The aim of this paper was to calibrate a model for the biological phosphorus removal process and to identify the causal micro-organisms. A model has been calibrated, indicating the necessity to account for low polyphosphate concentrations. The aerobic model was validated against different environmental conditions. It was confirmed that Acinetobacter is not a prerequisite for phosphorus removal. Representatives of the Enterobacteriaceae formed the most dominant group in the A^2/O system, while only 2% of the isolates of the SBR belonged to this group. Further identification of unidentified groups is necessary.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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