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ORIGINAL ARTICLE

Indigenous butyric acid-degrading bacteria as surrogate pit latrine odour control: isolation, biodegradability performance and growth kinetics

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Abstract

Butyric acid is one of the volatile organic compounds that significantly contribute to malodour emission from pit latrines. The purpose of this work is to isolate and identify bacterial strains that have the capability to degrade butyric acid, determine their butyric acid degradation efficiencies and estimate their growth pattern parameters of microbiological relevance. Pure cultures of bacterial strains capable of degrading butyric acid were isolated from pit latrine faecal sludge using an enrichment technique and were identified based on 16S rRNA analysis. The bacterial strains were cultured in mineral salt medium (MSM) supplemented with 1000 mg L^{-1} butyric acid, as a sole carbon and energy source, at 30 ± 1 °C, pH 7 and 110 rpm under aerobic growth conditions. The modified Gompertz model was used to estimate growth pattern parameters of microbiological relevance. Bacterial strains were phylogenetically identified as *Alcaligenes* sp. strain SY1, *Achromobacter animicus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Achromobacter xylosoxidans*, *Bacillus cereus*, *Lysinibacillus fusiformis*, *Bacillus methylotrophicus* and *Bacillus subtilis*. The bacterial strains in pure cultures degraded butyric acid of 1000 mg L^{-1} within 20–24 h. The growth kinetics of the bacterial strains in pure culture utilising butyric acid were well described by the modified Gompertz model. This work highlights the potential for use of these bacterial strains in microbial degradation of butyric acid for deodorisation of pit latrine faecal sludge. This work also contributes significantly to our understanding of bioremediation of faecal sludge odours and informs the development of appropriate odour control technologies that may improve odour emissions from pit latrines.

Keywords Biodegradation · Butyric acid · Growth kinetics · Odour · Pit latrine

Introduction

Simple latrines that safely contain faeces have been used in essence to eliminate open defecation. Pit latrines are the predominant means of human excreta collection for an estimated 1.77 billion people in low-income communities in the developing world (Graham and Polizzotto 2013). It is expected that there will be a burgeoning use of pit latrines in response to meeting the sanitation-related target of achieving Sustainable Development Goal (SDG) 6.2: universal access to safe sanitation by 2030 (Ravenscroft et al. 2017). While high pit latrine coverage levels are realised, of great concern is the fact that open defecation is obstinately continuing either by preference or necessity (Mara 2017). Surprisingly, some individuals even in households that own a working latrine, nevertheless, prefer to defecate in the open. Open defecation has long-since been implicated in the transmission of numerous infectious diseases and adverse health effects such as small-intestine bacterial overgrowth, diarrhoea, typhoid, giardiasis, soil-transmitted helminthiases, anaemia, environmental enteropathy and cholera. These are in addition to life threatening violence against women and girls (Jadhav et al. 2016; O'Reilly 2016).

Statistics in South Africa indicate that 4% of households still practise open defecation with the majority of the households living in the rural and informal settlements (STATSA 2016). Hutton and Chase (2016) found that this is due to contextual, technological and behavioural factors that are

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associated with sanitation adoption. Malodours that emanate from latrines are reported to be one of the impediments to investment, adoption and consistent use of pit latrines as shown by experiences in sanitation promotion in the developing countries (Rheinländer et al. 2013). Several studies (Grimason et al. 2000; Lundblad and Hellström 2005; Diallo et al. 2007; Le et al. 2012; Tsinda et al. 2013; Obeng et al. 2015) have alluded to the same. Moreover, while malodours are intrinsically not noxious, they can cause nausea, stress and annoyance to communities; in addition to its adverse effects on aesthetics and property values (Mills 1995; Rappert and Muller 2005). Malodours can also attract flies, which are the most important water- and excreta-related diseases carriers and spreaders (Morgan 2014; Nakagiri et al. 2016). In response to concerns about the detrimental effects of the offensive smells, which emanate from the pit latrines, people, including children and adults, abandon them in favour of alternatives to latrines including open defecation (Rheinländer et al. 2013).

Studies conducted by Chappuis et al. (2016) showed that butyric acid ($C_4H_8O_2$) is one of the four key odorants that significantly contribute to human faecal odour. Butyric acid is a four carbon short-chain fatty acid, which is one of the intermediate products of anaerobic digestion, in which complex soluble organic materials are reduced to a methane (CH_4) and carbon dioxide (CO_2) mixture as the main final products (Siegert and Banks 2005). This process comprises of a continuum of metabolic reactions (hydrolysis, acidogenesis and methanogenesis) as a result of a complex intimate relationship between the acid-forming species and the methane-producing species of bacteria (Lee et al. 2015). Butyric acid, in its pure state as an individual compound, exhibits an idiosyncratic smell of sweet rancid (Sheridan et al. 2003; Otten et al. 2004), which makes it offensive to handle. It is one of the volatile compounds that have a very low human odour detection threshold (Sheridan et al. 2003).

Over the years, there are many technologies and strategies that have been developed by the users as well as scientists to avert and mitigate malodours emission from the latrines. These include the following: use of naturally fragrance occurring substances, addition of wood ash, antiseptics, insecticides, lubricants, laundry and soapy water, motor-battery acids, detergents and modified latrine designs such as ventilated improved pit (VIP) latrine, urine-diverting dry and ecological sanitation toilets and pour flush latrines (Rheinländer et al. 2013). However, these strategies and technologies to a greater extent have not provided the desired results as they are associated with their own social, economic, institutional and technological challenges. The use of organisms for bioremediation of environmental pollutants, including odour-causing compounds, either in situ or ex situ, has lately been a subject of much research due to certain competitive advantages offered over the conventional physical and chemical treatment methods. Biological treatment is relatively efficient and cost-effective technology for environmental pollution attenuation, and uses microorganisms to reduce, oxidise or eliminate pollutants (Sheridan et al. 2003). Microorganisms capable of degrading malodorous compounds may be an attractive alternative to the existing odour control techniques and strategies currently used in low income settings in the developing world. However, detailed information on microorganisms that degrade odour-causing compounds, including butyric acid in the pit latrines, is scarce and very little is known about their degradation performance and growth behaviours.

In view of the above background, the objective of this work was to enrich, isolate and phylogenetically identify the indigenous bacterial strains from South Africa that have capabilities to utilise butyric acid as a sole source of carbon and energy and further determine their butyric acid degradation efficiencies. Also, the growth behaviour of the identified bacterial strains under studied environmental conditions was described by estimating their maximum specific growth rates, lag times and asymptotic values. To the best of our knowledge and after a thorough search in the literature, the use of aerobic bacteria isolated from pit latrine faecal sludge for degradation of butyric acid has not been reported in the literature yet.

Materials and methods

Chemicals and reagents

Analytical grade butyric acid (≥ 99% purity) was purchased from Sigma Aldrich Inc., St Louis, MO, USA. HPLC grade sulphuric acid (H_2SO_4) (98% purity) was purchased from Glassworld, South Africa. Other chemicals and reagents used in this study were of analytical grade and were locally purchased from Merck Chemicals (Pty) Ltd., Gauteng, South Africa.

Medium preparation

The mineral salt medium (MSM) consisted of the following: 2.722 g KH_2PO_4; 0.535 g NH_4Cl; 0.049 g $MgSO_4$; 4.259 g Na_2HPO_4; 0.114 g Na_2SO_4 per litre of 18.2 MΩ deionised water. The MSM was supplemented with 1 mL of trace element solution per litre of MSM solution. The trace element solution consisted of 0.0128 g $NiCl_2$, 0.549 g $CaCl_2$, 0.0124 g H_3BO_3, 6.9505 g $FeSO_4$, 0.0347 g $CuCl_2$, 0.0136 g $ZnCl_2$, 0.0103 g NaBr, 0.0121 g $NaMoO_2$, 0.0198 g $MnCl_2$, 0.0166 g KI and 0.0238 g $CoCl_2$ per litre of 18.2 MΩ deionised water (Roslev et al. 1998). For degradation and cell growth studies, MSM was supplemented with 1000 mg L^{-1} butyric acid. The pH of the medium was adjusted to 7.0, by titration with 6.0 M NaOH, which was prepared with 18.2 MΩ deionised water and sterilised by autoclaving at 121 °C for 15 min.

Faecal sludge sample collection and preparation

Faecal sludge samples were collected from pit latrines in the semi-rural mining area of Kendal, in Mpumalanga Province, South Africa at 26°5′24″S, 28°58′17 E. Pit latrines are the common means of human waste disposal, for the residents of the area. Faecal sludge samples were collected from a depth of 0 (surface) to 10 cm using pre-sterilised auger-like equipment. All non-faecal wastes (such as diapers, stones, clothes, metals, plastic bags, etc.) were removed. The samples were immediately transported to the laboratory and preserved at 4 °C prior to use.

A mass of approximately 100 g of faecal sludge sample was suspended in a pre-sterilised 2 L Schott bottle with 1 L of sterile 18.2 MΩ deionised water prepared by Purelab Flex purification system (ELGA Lab Water Ltd., UK). The mixture was vigorously vortexed for 5 min and the suspended solids were allowed to settle down for 10 min. The supernatant was subsequently filtered through sterilised cotton wool (Dischem, South Africa) in a sterilised funnel for complete removal of the top layer (scum). The cotton wool was replaced after every 100 mL of the supernatant is filtered to avoid cotton wool compacting when wet. The aliquot of the filtrate obtained therefrom was preserved at 4 °C prior to use for bacterial isolation.

Isolation and molecular identification of bacterial strains

A 1000 μL of an aliquot of the filtrate obtained from a mixture of faecal sludge and deionised water was subsequently inoculated into MSM supplemented with 500 mg L^{-1} butyric acid was incubated at 30 ± 1 °C on a temperature controlled rotary shaker at 110 rpm for 24 h in the dark. The procedure was repeated thrice to enrich microbial cultures and increase population density. Then, 100 μL of each resulting culture was serially diluted and was spread onto nutrient agar plate media and incubated for 24–48 h in the static incubator at 30 ± 1 °C in the dark. The strains were purified by streaking agar plates. Morphologically distinct colonies were streaked at least three times on fresh agar plates and incubated as above to obtain pure cultures in preparation for 16S rRNA sequence identification.

Bacterial genomic DNA was extracted using the boiling method from a 24–48-h pre-grown cell suspensions of the pure cultures. The 16S rRNA genes of isolates were amplified by a reverse transcriptase-polymerase chain reaction (RT-PCR). The amplification and sequencing was conducted by using universal forward primer (27F: 5′ GAG TTT GAT CCT GGC TCA G 3′) and reverse primer (1492R: 5′ GGT TAC CTT GTT ACG ACT T-3′). The RNA sequence analyses of the PCR products from the 16S rRNA gene of the isolates were obtained, submitted and compared with other genes available through the GenBank database using a basic BLAST of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). PCR amplification of the 16S rRNA yielded single fragments of 700 bootstraps based on 100 pseudo replicates. Phylogenetic dendograms were assembled based on the 16S rRNA gene sequence of isolates and closely related strains by neighbour-joining method using MEGA 6.0 (Tamura et al. 2013).

Degradation and bacterial growth conditions

To investigate the degradation of butyric acid as well as the growth of bacterial strains with butyric acid as a carbon and energy source, 1 mL of bacterial strain pure seed culture (OD_{600} = 2.0) (equivalent biomass, mg L^{-1}, for each of the bacterial strains are provided in Table S1 of the supplementary material (SM) was inoculated into 150 mL each of the MSM supplemented with 1000 mg L^{-1} butyric acid in 250 mL Erlenmeyer flask in triplicates. The experiments were aseptically conducted. Likewise, abiotic MSM with the same concentration of butyric acid was used as a control in triplicates. All the reactors were incubated at 30 ± 1 °C on a temperature-controlled rotary shaker in the dark at 110 rpm for 24 h. The samples were aseptically withdrawn at regular time intervals of 4 h to determine both butyric acid concentration and optical density (OD). Samples for determination of bacterial growth were withdrawn from the reactor before and at 4, 8, 12, 16, 20 and 24 h after starting incubation while for determination of butyric acid concentration, samples were withdrawn at 4, 8, 12, 16, 20 and 24 h after starting the incubation. From this procedure, the degradation efficiencies of the bacterial strains were determined with Eq. (1) (Gutarowska et al. 2014):

$$D_e = \left(\frac{A_c - A_s}{A_c}\right) \times 100\% \quad (1)$$

where D_e, A_c and A_s are the degradation efficiency of butyric acid (%), the concentration of butyric acid (mg L^{-1}) in the abiotic culture at t_n (0, 4, 8, 12, 16, 20 and 24 h) and the concentration of butyric acid (mg L^{-1}) in the biotic culture at t_n (0, 4, 8, 12, 16, 20 and 24 h), respectively.

Determination of bacterial growth kinetic parameters

Bacterial growth curve analysis was performed based on the modified Gompertz model to estimate the bacterial growth kinetic parameters of each of the bacterial strains. The modified Gompertz model has the form expressed according to the Eq. (2) (Gibson et al. 1988):

$$LogN_{(t)} = A + C.\exp\{-exp[-b(t-m)]\} \quad (2)$$

where $Log\ N_{(t)}$ is the decimal logarithm of optical density at time, t (h), A is the optical density value of the lower asymptote (dimesionless), C is the difference in optical density between inoculum and the stationary phase (dimensionless), m is the time at which the absolute growth rate is maximal (time at inflexion) (h), and b is the relative maximum growth rate determined at time, $m.(h^{-1})$ (the slope of tangent to the curve at m).

In this study, the relative optical density $N_{(t)}/N_{(0)}$ or $A_{(t)} - A_{(0)}$ was used for the densimetric assay. In view of this, the parameter, A, in Eq. (2) is equal to zero. Equation (2) subsequently changes to:

$$In\frac{N_{(t)}}{N_{(0)}} = C.exp\{-exp[-b(t-m)]\} \tag{3}$$

where $N_{(0)}$ is initial optical density at the time of inoculation, $t(h) = 0$ and C is the upper asymptotic value (dimensionless).

Similarly, Zwietering et al. (1990) described the modified Gompertz function as:

$$Y_{(t)} = a.exp[-\exp(b-ct)] \tag{4}$$

where $Y_{(t)}$ is $In\frac{N_{(t)}}{N_{(0)}}$.

The Zweitering's parameter, a, is the same as the Gibson's parameter, C; therefore, the Eq. (3) can be written as (Garthright 1991):

$$Y_{(t)} = a.exp\{-exp[-b(t-m)]\} \tag{5}$$

The Gompertz function does contain mathematical parameters; a, b and m rather than parameters of biological meaning asymptote value, A (dimesionless), maximum growth rate, μ_m (h^{-1}), and lag time, λ (h). Additionally, it is easy to compute the biological parameters with 95% confidence intervals if they are directly computed from the mathematical parameters in the Eq. (5). Hence, an expression of biological parameters was derived as a function of the parameters of the basic function as follows:

The maximum specific growth rate (μ_m), Eq. (6) was derived as follows:

$$\mu_m = \frac{b.a}{e} \tag{6}$$

where asymptote value, a, in Eq. (7) is reached for time is approaching infinity and is expressed as:

$$A = a \tag{7}$$

and the lag time, λ, Eq. (8) was derived as follows:

$$\lambda = m - \frac{1}{b} \tag{8}$$

By substituting the biological parameters in Eq. (5), the re-parameterised modified Gompertz models can be written as (Zwietering et al. 1990; Mytilinaios 2013):

$$y = A.exp\left\{-exp\left[\frac{\mu_m - e}{A}(\lambda - t) + 1\right]\right\} \tag{9}$$

The model was iteratively best fitted to the experimental data by Levenberg Marquardt based on non-linear least-squares *a*lgorithms through minimisation of the sum of the squares of the errors between the model and the experimental data points by adaptively varying the parameter values between the Gauss-Newton update and the gradient descent update (Garvin 2017). The non-linear curve fitting was successfully achieved using Origin 2018 data analysis and graphing software (Originlab Corporation, Northampton, MA, USA) with $\alpha = 0.005$ for all the parameters. Each growth curve was generated based on the average of experiments carried out in triplicates. The fitted curves were statistically evaluated using the coefficient of determination (R^2) and root mean square error (*RMSE*) as expressed by Eq. (10) and Eq. (11), respectively:

$$R^2 = 1 - \frac{\sum e_i^2}{\sum\left(y_i - \overline{y}\right)} \tag{10}$$

where e_i is an error of the predictive values, y_i is the predicted values and $\overline{y}$ is mean of the predicted values.

$$RMSE = \sqrt{\sum_{i=1}^{N}(E_i - O_i)^2} \tag{11}$$

where E is the predicted value and O is the observed value.

Analytical procedures

The aliquot (6 mL) of culture medium was withdrawn from the enrichment flasks at 4 h time intervals and was centrifuged at 9000 rpm for 10 min at room temperature, using a Minispin centrifuge of Eppendorf AG type (Hamburg, German). The supernatant was subsequently filtered through Milipore Millex-GV Hydrophilic PVDF 0.22 μm membrane and dispersed into 2 mL HPLC vial prior to analysis.

All analyses in this work were performed on a Waters Alliance 2695 Separation Module HPLC system (Waters Corporation, Milford, MA, USA) in triplicates to determine the residual butyric acid concentration. The system was equipped with a low-pressure mixing pump, an inline degasser, an auto-sampler with programmable temperature control (samples held at 5 °C) and a Waters 2998 Photodiode array detector (PAD) equipped with micro UV cell (Waters Corporation, Milford, MA, USA). An HPLC mobile phase of 0.02 M sulphuric acid (H_2SO_4) was used. The mobile phase was prepared by diluting 1.1 mL of 18.4 M H_2SO_4 with

18.2 MΩ deionised water to a final volume of 1.0 L. This was filtered through a Nylon 5-μm membrane before injection into the HPLC. Sample injection volume of 10 μL was used for all analyses. The stationery phase was an Aminex HPX-87H87H ion-exclusion organic acid, 300 mm × 7.8 mm, 9 μm particle size column (Bio-Rad Laboratories, Berkeley, CA, USA) ran with an isocratic flow rate of 1 mL min^{-1} at a column temperature of 60 °C. The detection of the peaks was achieved at a wavelength of 210 nm. Retention time for butyric acid was 12.2 min and the total run time was set at 15 min. Chromatographic data were processed by Empower2 Build 2154 software (Waters Corporation, Milford, MA, USA). Qualitative and quantitative data were obtained by comparing the peak area and peak height to butyric acid standard compound with known concentration. The concentration of butyric acid was deduced from an external calibration curve.

Quantitative determination of bacterial growth yields that was determined in the medium was spectrophotometrically monitored by measuring the OD at a single wavelength λ = 600 nm using a UV Lightwave II spectrophotometer (Labotec, Gauteng, South Africa). The quartz cuvette of 10 mm optical path length was used to carry the aliquots in the sample chamber of the spectrophotometer. The measurements were blanked to zero using the same MSM without inoculum as a reference. All the experiments (both biotic and abiotic) were performed in triplicates. The dry weight method was applied to estimate biomass in milligram per litre. The generated calibration equations of each bacterial strain are listed in Table S1 of the SM.

Results and discussion

Isolation and molecular identification of the bacterial strains

In this study, indigenous aerobic bacterial strains capable of utilising butyric acid as a sole carbon and energy source were successfully isolated from pit latrine faecal sludge. There were a total of 24 morphologically distinct bacterial colonies that were isolated. The isolates were further screened for their butyric acid-degrading ability using MSM supplemented with butyric acid. Of the 24 bacterial isolates tested, 9 bacterial isolates demonstrated pronounced growth in butyric acid-supplemented MSM as pure cultures after enrichment and purification. The bacterial isolates were designated as Ba, B1a, B1b, B6a, B5a, B7a, C4c, CrNb and CrNc for identification purposes. The RNA sequence analyses of the PCR products from the 16S rRNA gene of the isolates were obtained, submitted and compared with other genes in GenBank using a basic BLAST of the NCBI. The 16S rRNA gene sequencing was used for identification because it is present in virtually all bacteria and its role has not temporarily changed (Garcha et al. 2016). Further, the identification is more objective as optimal growth and microbial viability are not the prerequisites (Reller et al. 2007). Comparative phylogenetic dendrograms generated based on 16S rRNA gene sequences of the isolates with closely related species revealed that the bacterial isolates Ba, B1a, B1b, B6a, B5a, B7a, C4c, CrNb and CrNc clearly marched with *Alcaligenes* sp. strain SY1, *Achromobacter animicus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Achromobacter xylosoxidans*, *Bacillus cereus*, *Lysinibacillus fusiformis*, *Bacillus methylotrophicus* and *Bacillus subtilis*, respectively. Their phylogenetic dendrograms showing the closest NCBI (BLASTn) relatives based on the 16S rRNA gene sequence were constructed by the neighbour-joining method as shown in Fig. 1.

The highest sequence homology (% identity) of each bacterial strain and their closely related strains are also presented in Table 1. The identification of the high percentage of *Bacillus* sp. related strains is probably because *Bacillus* strains are not difficult to cultivate in the medium used in this study, or environmental conditions in the pit latrines in Kendal, South Africa are favourable for their survival and growth (Zhang et al. 2010). To the best of our knowledge and after thorough search in the literature, this is the first time all these bacterial strains but members of genus *Pseudomonas* have been reported to utilise butyric acid as the sole carbon and energy source (Sheridan et al. 2003) and *Bacillus* sp. in a mixture of other volatile fatty acids (VFAs) (Yun and Ohta 1997). Since they are indigenous organisms, they are more likely to survive and to be active than exogenous bacterial strains when introduced into pit latrine environments in South Africa or similar environments. The introduced exogenous bacterial strains are more likely to be subjected to intense competition, predation or parasitism after their release into the target environment (Han et al. 2015), in this case the pit latrine.

Butyric acid degradation by pure bacterial cultures

The ability of the bacterial strains to utilise butyric acid as a sole source of carbon and energy was investigated. As shown in Fig. 2, the initial 1000 mg L^{-1} of butyric acid can be biodegraded effectively by the indigenous pure bacterial strains as it can be observed that it was completely degraded within 20–24 h. However, the degradation rates varied from one bacterial strain to another. The bacterial strains *Achromobacter xylosoxidans*, *Bacillus subtilis*, *Lysinibacillus fusiformis*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Bacillus methylotrophicus* completely degraded 1000 mg L^{-1} butyric acid within 20 h while *Achromobacter animicus*, *Serratia marcescens* and *Alcaligenes* sp. strain SY1 completely degraded butyric acid within 24 h. The reason for the differences in degradation efficiencies is unclear.

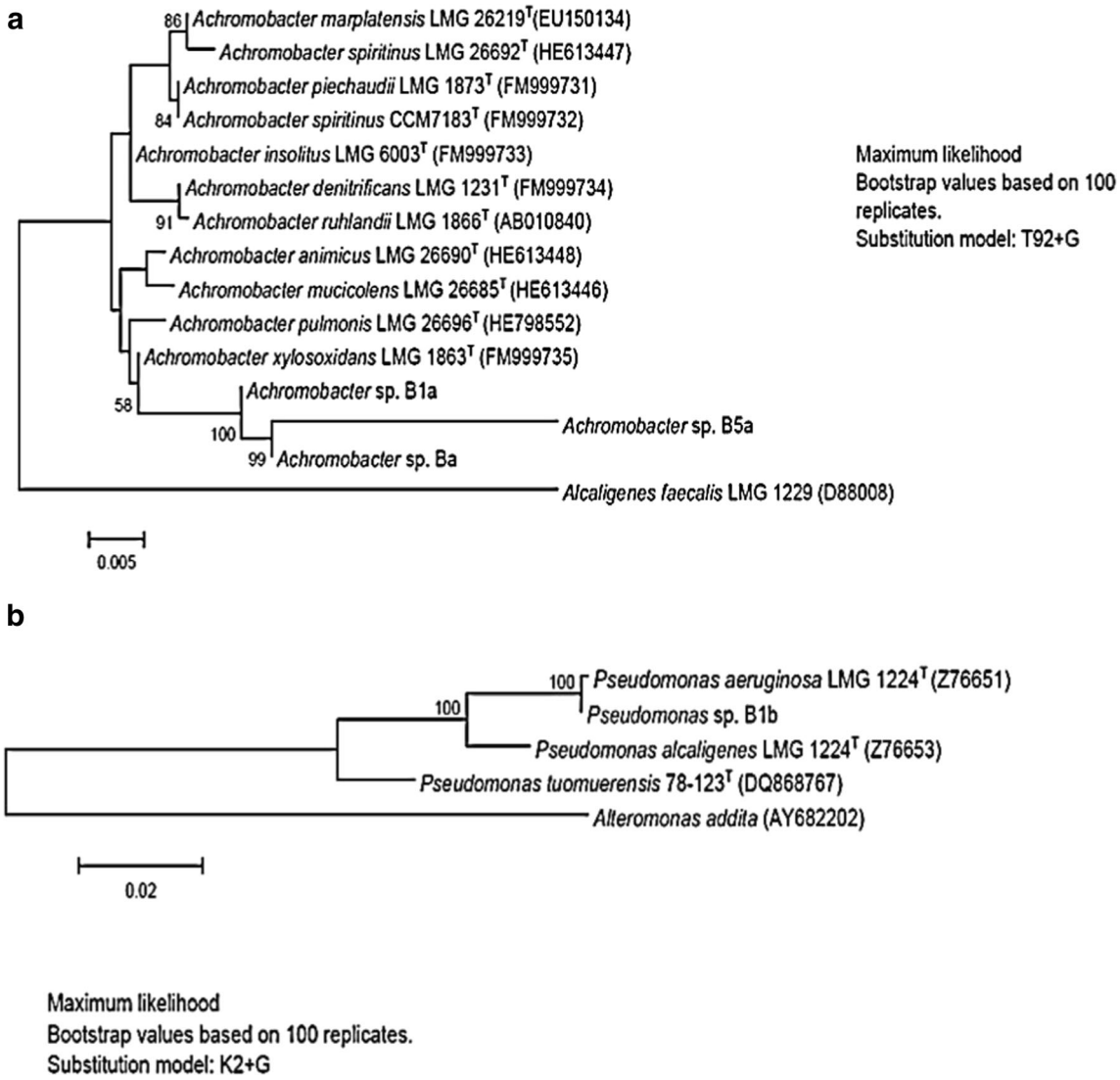


Fig. 1 **a** The phylogenetic tree for *Alcaligenes* sp. SY1, *Achromobacter animicus* and *Achromobacter xylosoxidans* and related strains based on 16S rRNA gene sequences. Bootstrap values were based on 100 replicates. **b** The phylogenetic tree for *Pseudomonas aeruginosa* and related strains based on 16S rRNA gene sequences. Bootstrap values were based on 100 replicates. **c** The phylogenetic tree for *Serratia marcescens* and related strains based on 16S rRNA gene sequences. Bootstrap values were based on 100 replicates. **d** The phylogenetic tree for *Bacillus cereus* and related strains based on 16S rRNA gene sequences. Bootstrap values were based on 100 replicates. **e** The phylogenetic tree for *Bacillus methylotrophicus*, and *Bacillus subtilis* and related strains based on 16S rRNA gene sequences. Bootstrap values were based on 100 replicates. **f** The phylogenetic tree for *Lysinibacillus fusiformis* and related strains based on 16S rRNA gene sequences. Bootstrap values were based on 100 replicates

Previous studies (Bourque et al. 1987; Yun and Ohta 1997; Chin et al. 2010) have found that many bacterial strains can degrade butyric acid. For instance, Bourque et al. (1987) isolated *Acinetobacter calcoaceticus*, *Alcaligenes faecalis* and *Arthrobacter flavescens* from swine waste that was able to aerobically degrade butyric acid completely in the presence of other VFAs such as acetic acid, propionic acid, isobutyric acid and valeric acid and phenol and *p*-cresol after incubation at 29 °C and 200 rpm within 3 to 5 days. Yun and Ohta (1997) isolated bacterial strains identified as *Bacillus* sp., *Rhodococcus* sp. and *Staphylococcus* sp. from seed culture which was used for the treatment of animal faeces which exhibited growth on butyric acid in the presence of other VFAs after incubation of 37 °C and medium pH of 8.0 for 2 days. Conversely, in these previous studies, butyric acid was not the sole source of carbon. Only Chin et al. (2010) isolated bacterial strains identified as *Acinetobacter calcoaceticus*, *Wautersia paucula*, *Burkholdeira cepacia* which have the ability to completely degrade 1000 mg L^{-1}butyric acid as a sole source of carbon and energy. The complete degradation of butyric acid was achieved within 18 h for *Acinetobacter calcoaceticus* while the other strains it was achieved within 30–55 h at 30 ± 1 °C and pH 7.0.

The complete degradation of butyric acid in this work is important. This is primarily due to the fact that even at low

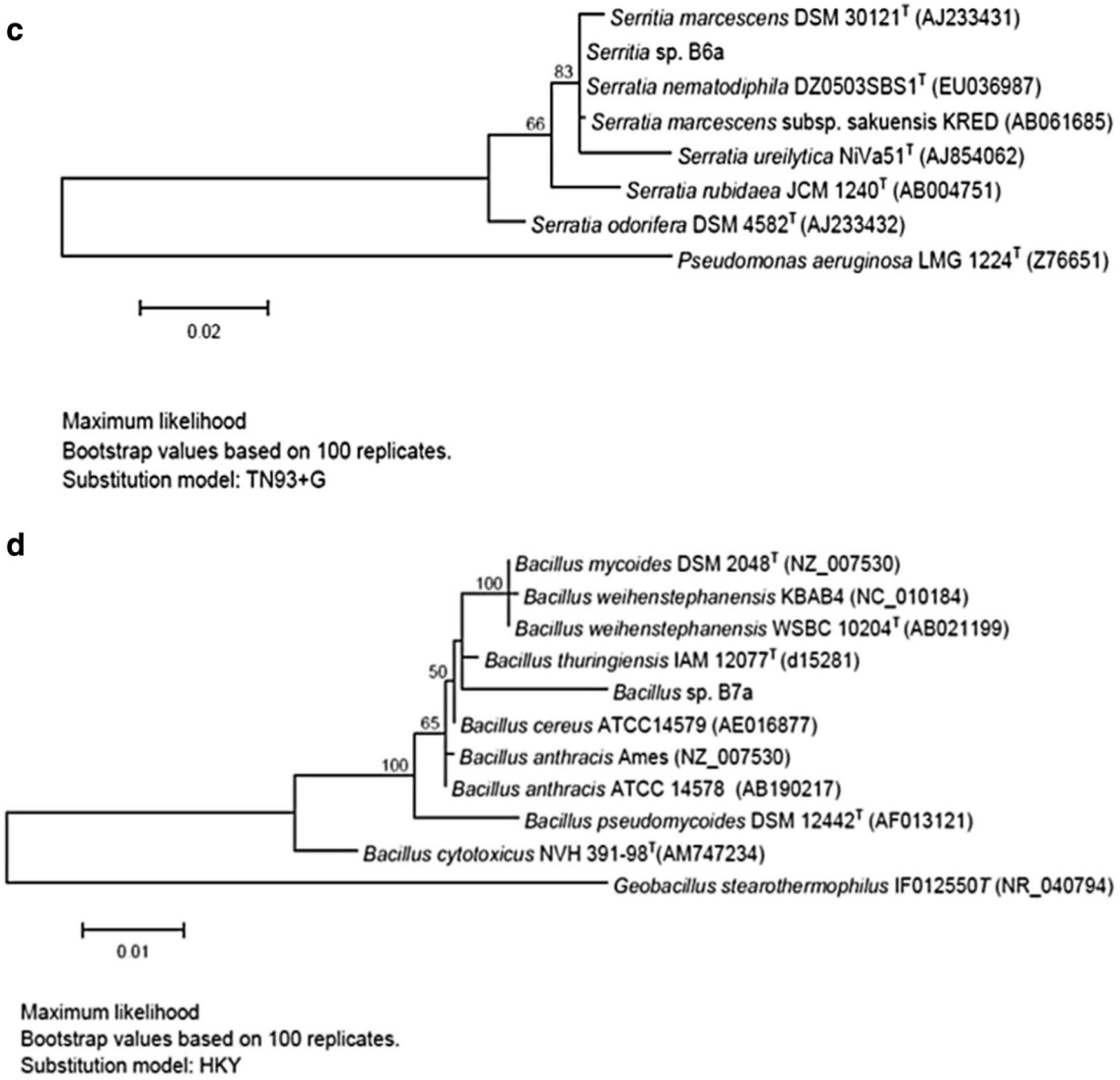


Fig. 1 continued.

concentrations, butyric acid is one of the VFAs that has high odour nuisance index. Its odour can even create problems at a receptor of odour nuisance at distances far away from the points of emission. This is attributed to its very low odour detection threshold (Sheridan et al. 2003). Butyric acid is one of the short-chain volatile fatty acids (SVFAs) which infinitely dissolves in aqueous solution (Hughes 1934). Hence, the high degradation of butyric acid could be attributed to its high rates of dissolution and solubility in water which determines its bioavailability (Kristiansen et al. 2011).

As shown in Fig. 2, in the control experiments, the concentration of butyric acid remained almost stable from 1000 to 996.99 mg L^{-1} during the incubation for 24 h. The loss of butyric that resulted from abiotic process was insignificant. This could be attributed to either surface volatilisation losses or photo-degradation due to exposure to light during sample withdrawals that was inevitable.

The butyric acid degradation and growth potential of the bacterial strains were investigated in detail. Although it was not known that these are their optimal growth conditions, all the strains showed remarkable ability to grow well at pH 7.0, 30 ± 1 °C and agitation rate of 110 rpm and butyric acid concentrations 1000 mg L^{-1} utilising butyric acid as the growth substrate as provided as a sole source of carbon and energy with initial seed culture of 2.0. The increase in cell density of each bacterial strain as expressed by its absorbance value measured at 600 nm was positively correlated to degradation efficiency of butyric acid as illustrated in Fig. 2. The Pearson correlation coefficients were in the range of 0.990 (*Achromobacter animicus*) to 0.999 (*Lysinibacillus fusiformis*) at $p < 0.01$. Bacterial cell density was increased with incubation time in all the bacterial strains, reaching the maximal density at different times that ranged from 0.990 ± 0.01 to 1.25 ± 0.004 within 20 to 24 h dependant on the bacterial strain as can be seen in Fig. 3.

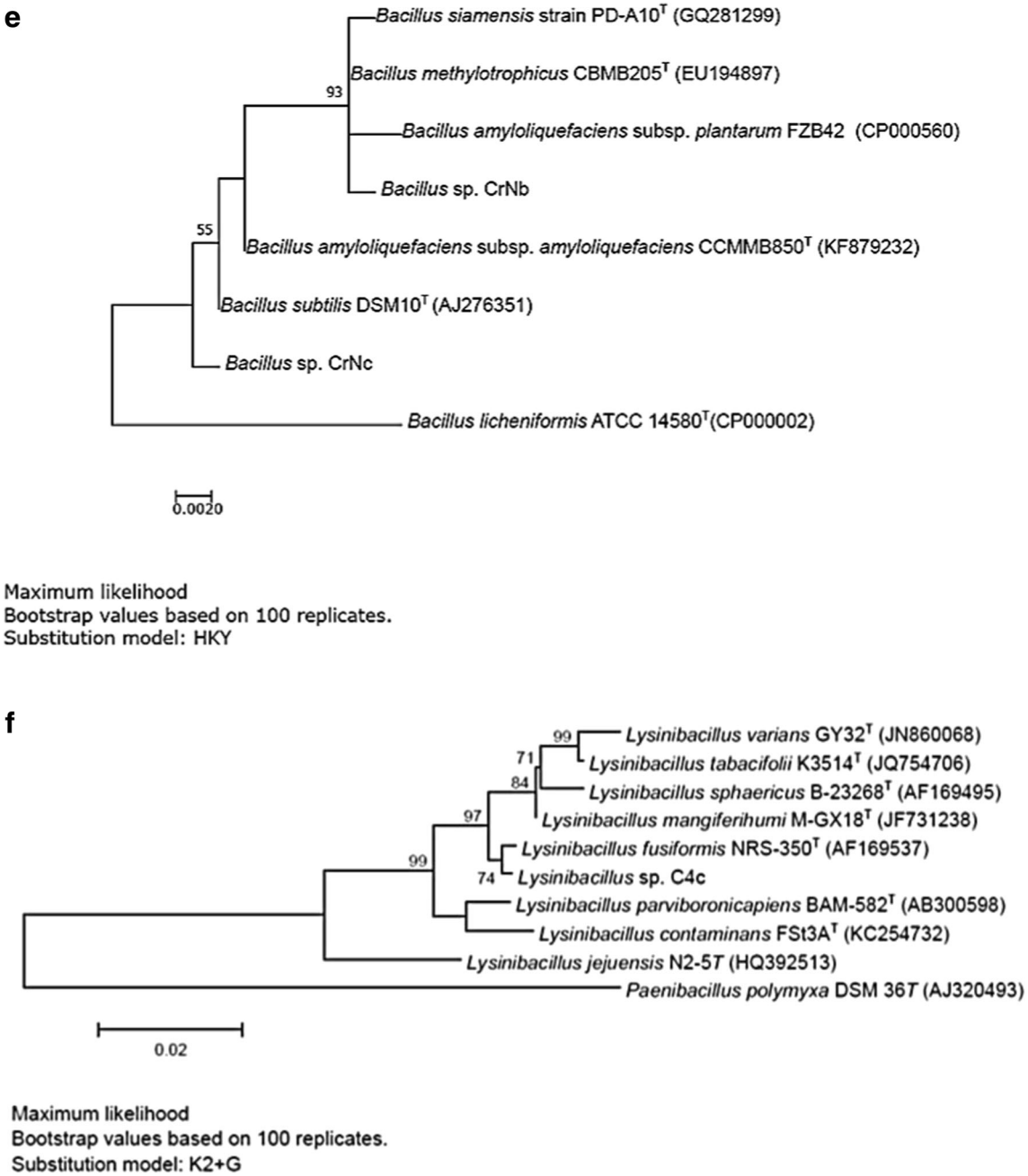


Fig. 1 continued.

Butyric acid was degraded by all the bacteria strains as shown in Fig. 2 and Fig. 3. However, during the lag phase particularly 4 h after incubation, all the bacterial strains but *Achromobacter xylosoxidans* and *Bacillus cereus* did not manifest the degradation of butyric acid as determined by the HPLC. It is assumed that this lag phase allows the bacteria to adapt to the new environmental conditions required for bacterial cells to begin cell division (Baranyi et al. 1993). Although there were variations in degradation efficiencies of butyric acid between bacterial strains during the duration of the lag phase, the high degradation efficiencies were observed in the exponential phase of growth for all the bacterial strains. Thus, 95 to 100% of the butyric acid degradation occurred in this phase. Generally, there was a very high increase in butyric acid degradation efficiencies of the bacterial strains near the mid-exponential growth phase and decreased as the cultures aged towards the early stationary phase. This is consistent with Kotler et al. (1993) previous observations that bacterial cells in their exponential growth phase rapidly consume the available nutrients in most nutritionally defined media and then ceases to grow exponentially.

Table 1 Closest relatives of the 16S rRNA gene sequences of bacterial isolates in this study

	Isolate designation	Closest hit	Accession no.	Homology (%)
1	Ba	*Alcaligenes* sp. strain SY1		99
2	B1a	*Achromobacter animicus* LMG26690^T	HE613448	99
3	B1b	*Pseudomonas aeruginosa* LMG 1224^T	Z76651	100
4	B5a	*Achromobacter xylosoxidans* LMG 26686^T	FM999735	93
5	B6a	*Serratia marcescens* DMS 30121^T	AJ233431	100
6	B7a	*Bacillus cereus* ATCC14579	AE016877	100
7	C4c	*Lysinibacillus fusiformis* NRS-350^T	AF169537	100
8	CrNb	*Bacillus methylotrophicus* CBMB205^T	EU194897	100
9	CrNc	*Bacillus subtilis* DSM10^T	AJ276351	100

Kinetics of bacterial growth

The biodegradation of butyric acid in batch reactors led to the formation of biomass. The amount of biomass formed increased with the degradation of butyric acid as observed in Fig. 3 but increased exponentially with respect to time during the log phase. Further, the increase in biomass concentration was dependent on the concentration of butyric acid remaining in the solution. Due to inadequate knowledge about the structural connectivity and functional mechanisms of the systems of the bacterial strains at the physiological level, an empirical model was used to understand the primary system purely based on its extrinsic behaviour. Numerous mathematical models and equations that describe microbial growth in culture media have been developed and used. These include Gompertz, Logistic, Richards, Stannard, Schnute models, etc. (Longhi et al. 2017). These models are numerically easier to handle as opposed to mechanistic models (Thakur 1991), for instance, the Monod and Michaelis-Menten based models which are preferred for systems to be scaled-up consistently.

In this work, based on a modified Gompertz model (Eq. (5)) mathematical parameters, *a*, *b* and *m* for bacterial growth were predicted. The model described the growth kinetics of all the bacterial strains individually as pure cultures from the lag phase to the stationary phase (Baty and Delignette-Muller 2004). The parameters of biological meaning such as lag time (λ), maximum specific growth rate (μ_m) and asymptotic growth level (*A*) as shown in Table 2 were also calculated by fitting the model parameters to the experimental data. This was founded on Eq. (2) to Eq. (9) as derived by Zwietering et al. (1990) as previously

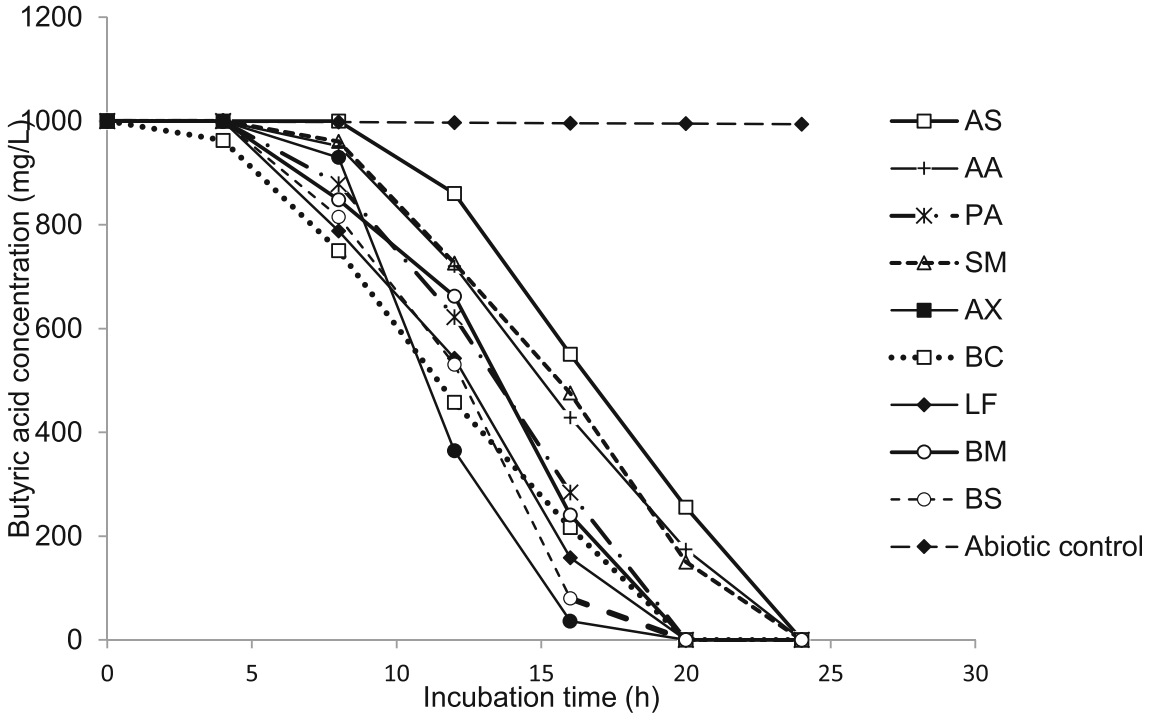


Fig. 2 Butyric acid degradation kinetics by different bacterial isolates; *Alcaligenes* sp. strain SY1 (AS), *Achromobacter animicus* (AA), *Pseudomonas aeruginosa* (PA), *Serratia marcescens* (SM), *Achromobacter xylosoxidans* (AX), *Bacillus cereus* (BC), *Lysinibacillus fusiformis* (LF), *Bacillus methylotrophicus* (BM) and *Bacillus subtilis* (BS)

discussed in the materials and methods section above. The model was chosen because it has a term of time delay introduced which allows it to fit a sigmoidal pattern of growth, which is an analogous pattern most bacteria follow as noted in most published research work. This is unlike the classical Gompertz model which does not take into consideration the delay time (Mytilinaios et al. 2012). Further, the model was re-parameterised in such a way that those parameters such as μ_m, λ and A, that are microbiologically significant, can be more suitably estimated (Zwietering et al. 1990). It is, therefore, viewed as the best sigmoidal model that describes bacterial growth data both in terms of statistical accuracy and simplicity in use as opposed to analogous sigmoidal models (Baty and Delignette-Muller 2004).

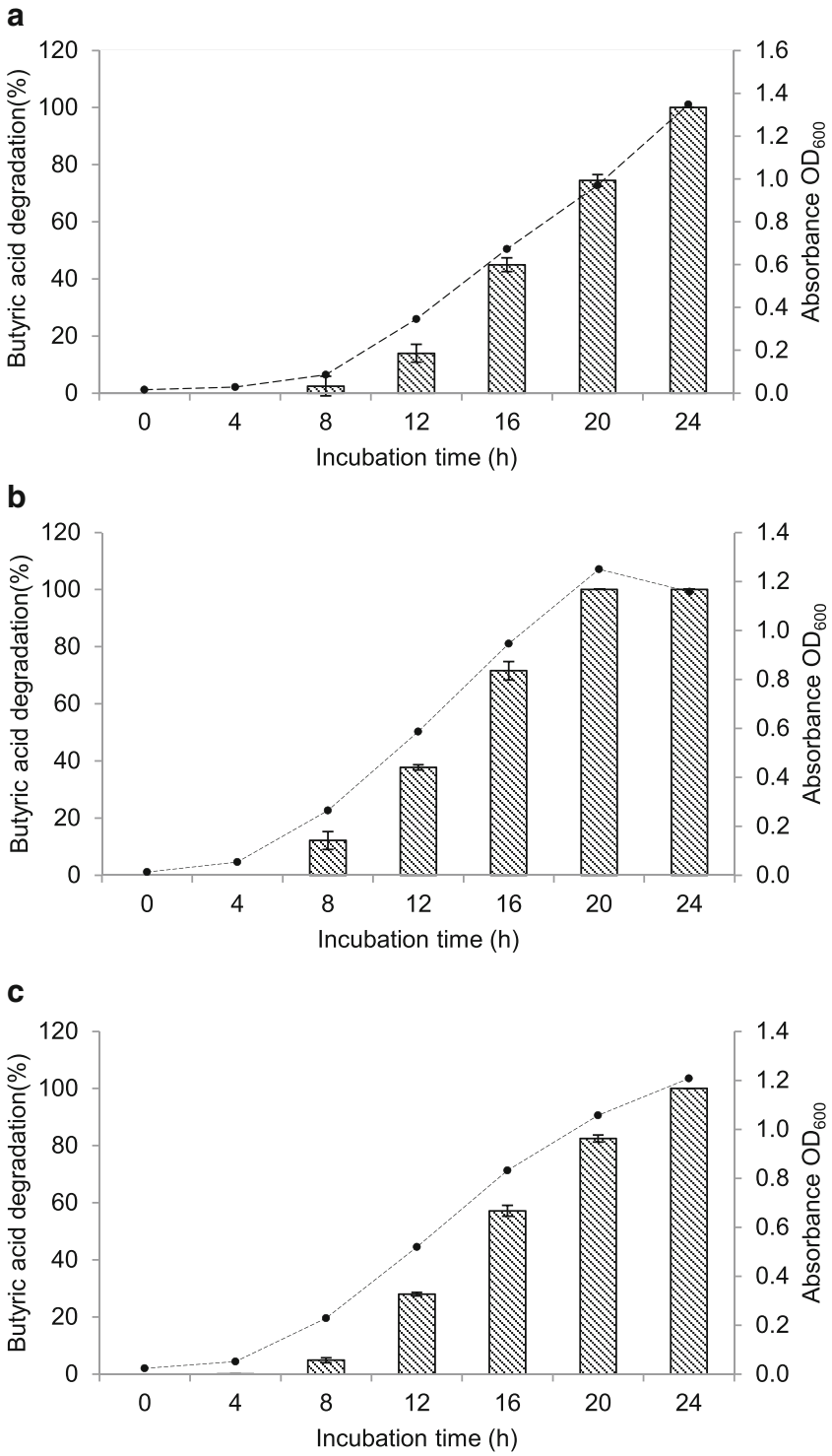
The OD measurements were used for estimation of growth parameters due to the merits of the method over conventional viable counts methods. It is considered to be rapid, non-destructive, relatively inexpensive and easy to automate method to monitor bacterial growth (Dalgaard and Koutsoumanis 2001; Perni et al. 2005). Actually, the OD measurements have recently been used to accurately derive growth parameters using numerous techniques and mathematical models (Dalgaard and Koutsoumanis 2001; Koseki and Nonaka 2012; Pla et al. 2015). However, growth rates of relatively high cell density cultures are those that can be determined directly from the changes in OD measurements (Dalgaard and Koutsoumanis 2001).

The average growth kinetic parameters for each of the nine bacterial strains exposed to the same experimental conditions and with the same preculture history and standardised inoculum are shown in Table 2. When the growth parameters were compared, the lag time was in the range of 5.54 h (*Achromobacter animicus*) and 8.47 h (*Alcaligenes* sp. SY1). The maximum specific growth rate was between 0.07 h^{-1} (*Serratia marcescens*) and 0.15 h^{-1} (*Achromobacter xylosoxidans*). The values of the parameters might be overestimated as the model is known for overestimation of lag time and maximum specific growth rate as one of the major drawbacks to its use (Baty and Delignette-Muller 2004). The maximum biomass concentration was between 1.06 (*Bacillus subtilis*) and 1.59 (*Alcaligenes* sp. SY1). It is worth stating that the growth curves for *Alcaligenes* sp. SY1, *Achromobacter animicus*, and *Serratia marcescens* did not reach the stationary phase; therefore, their asymptotic growth levels predicted by the model could be estimated with uncertainty which might affect the values of the other parameters (Longhi et al. 2017).

The model estimated the expected values for the growth parameters and fitted the data well, as demonstrated by the analysed statistics. As can be seen in Table 2, according to goodness-of-fit criterion, the coefficient of determination, R^2 (Eq. (10)), to evaluate fitting of the modified Gompertz model, was found to be high ranging between 0.986 and 0.999. The R^2 is a statistical measure of the proportion of the variability in the data set, which is used to predict a response using the model (Sant'Ana et al. 2012). The high R^2 values for the model obtained in this work suggest that the modified Gompertz model

Fig. 3 **a** Butyric acid degradation and bacterial growth under pH 7, 30 ± 1 °C and 110 rpm against incubation time of *Alcaligenes* sp. strain SY1: butyric acid degradation efficiency, bar graphs; bacterial growth, line graph. **b** Butyric acid degradation and bacterial growth under pH 7, 30 ± 1 °C and 110 rpm against incubation time of *Pseudomonas aeruginosa*: butyric acid degradation efficiency, bar graphs; bacterial growth, line graph. **c** Butyric acid degradation and bacterial growth under pH 7, 30 ± 1 °C and 110 rpm against incubation time of *Achromobacter animicus*: butyric acid degradation efficiency, bar graphs; bacterial growth, line graph. **d** Butyric acid degradation and bacterial growth under pH 7, 30 ± 1 °C and 110 rpm against incubation time of *Achromobacter xylosoxidans*: butyric acid degradation efficiency, bar graphs; bacterial growth, line graph. **e** Butyric acid degradation and bacterial growth under pH 7, 30 ± 1 °C and 110 rpm against incubation time of *Serratia marcescens*: butyric acid degradation efficiency, bar graphs; bacterial growth, line graph. **f** Butyric acid degradation and bacterial growth under pH 7, 30 ± 1 °C and 110 rpm against incubation time of *Bacillus methylotrophicus*: butyric acid degradation efficiency, bar graphs; bacterial growth, line graph. **g** Butyric acid degradation and bacterial growth under pH 7, 30 ± 1 °C and 110 rpm against incubation time of *Bacillus cereus*: butyric acid degradation efficiency, bar graphs; bacterial growth, line graph. **h** Butyric acid degradation and bacterial growth under pH 7, 30 ± 1 °C and 110 rpm against incubation time of *Lysinibacillus fusiformis*: butyric acid degradation rate, bar graphs; bacterial growth, line graph. **i** Butyric acid degradation and bacterial growth under pH 7, 30 ± 1 °C and 110 rpm against incubation time of *Bacillus subtilis*: butyric acid degradation rate, bar graphs; bacterial growth, line graph

prediction adequately described the bacterial strains' growth curves of the observed experimental data. Root mean square error, *RMSE* (Eq.(11)), is a standard statistical measure of the precision of a predictive model, and gives an explanation for the differences between predicted and observed values (Sant'Ana et al. 2012). The RMSE values in Table 2 to validate the model's performance revealed that it provided a reliably better goodness-of-fit to the observed experimental data for all bacterial strains. By comparing the statistical criterion of RMSE values of the modified Gompertz for all bacterial strains' growth curves, the results show that *Achromobacter animicus* growth curve had the smallest RMSE value (0.0002) while *Bacillus methylotrophicus* had the highest RMSE value (0.004). This demonstrated that of all the bacterial strains in this work, the modified Gompertz model adequately described the growth of *Achromobacter animicus* at the set environmental conditions. Comparatively, these RMSE values are significantly smaller than some that have been reported in the literature. For instance, Pla et al. (2015) checked the performance of different primary models (three-phase linear and non-linear; Gompertz, Logistic, Richards and Baranyi) in their modified forms to describe OD growth curves of *Bacillus cereus*, *Listeria monocytogenes* and *Escherichia coli*. Although their R^2 (0.939–0.999) were close to those calculated in this work, their RMSE values (0.007–0.061) were much higher than those calculated in this work. The smaller RMSE values obtained essentially reveal the suitableness of the model in this work. This connotes that the estimated growth parameters for the bacterial strains estimated on the basis of OD measurements in this work can be more appropriately evaluated.



The modified Gompertz model used in this study appears to satisfactorily fit the bacterial strains' growth curves as shown in Fig. S1 of the SM. However, in contrast with other or analogous empirical sigmoidal growth models reported in literature, different studies have reached different conclusions. Pla et al. (2015) studied the growth of *Bacillus cereus* at 30 °C in brain heart infusion (BHI) medium with different inoculum concentrations and shown that the Richards model had best statistical goodness-of-fit in predicting its growth parameters using optical density growth curves. When these authors (Pla et al. 2015) in the same study used plate count growth curves, the observations were dissimilar, the Baranyi model was the best fitting model. Similarly, in their study, Tarlak and colleagues (Tarlak et al. 2018) concluded that modified Baranyi model gave better goodness-of-fit than the modified logistic and Gompertz models in describing the growth behaviour of *Pseudomonas*

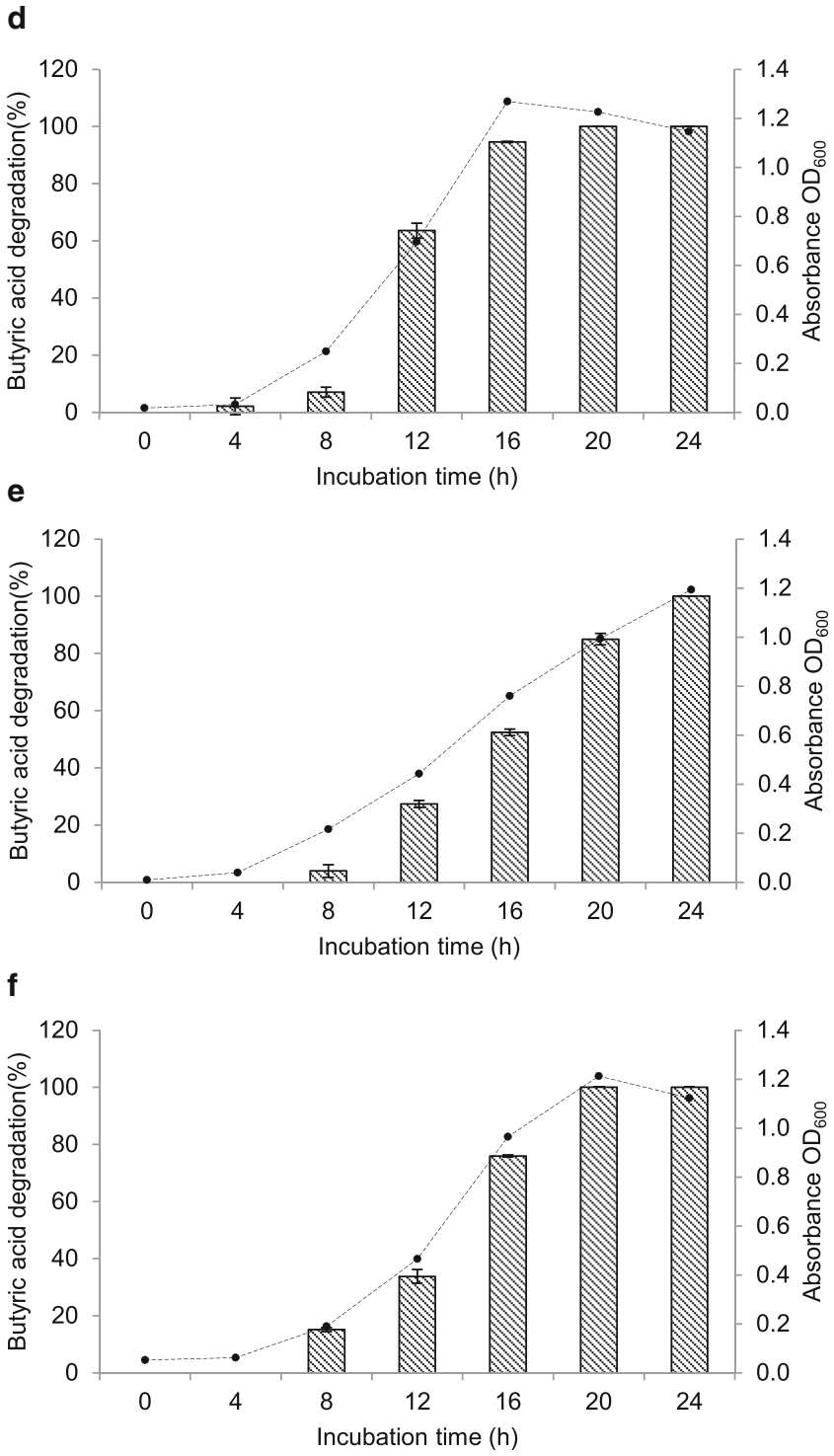


Fig. 3 continued.

spp. on the sliced mushroom at different isothermal storage temperatures. In contrast, Li et al. (2013) reported that the mean values of four statistical criteria showed that the modified Gompertz model adequately described the growth of *Pseudomonas* spp. in pallet-package pork under isothermal conditions at different temperatures, although they noted that the modified Gompertz, Baranyi and Huang models could not give a consistently better goodness-of-fit over all the growth curves at all different temperatures. Also, Zwietering et al. (1990) reported that the growth data of *Lactobacillus plantarum* incubated at different temperatures in MRS medium were better fitted with the Gompertz model compared to linear, quadratic, exponential, logistic and t^{th}power models. Furthermore, George et al. (1996) studied the combined effect of different temperatures, pH values

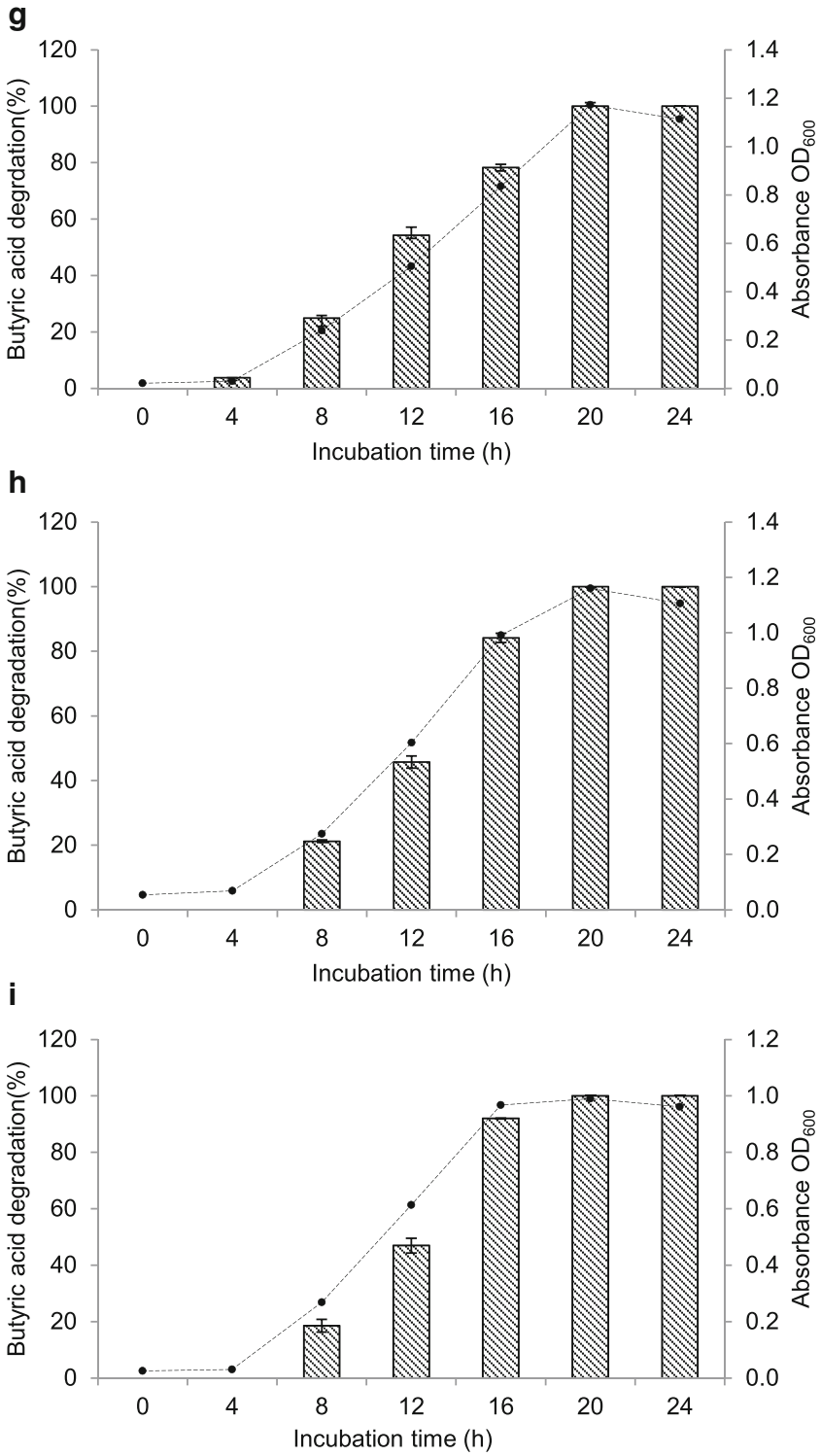


Fig. 3 continued.

and acetic and lactic acids on the growth of *Listeria monocytogenes*. They concluded that the Baranyi model provided the best fitting for the growth data. Qi et al. (2006) indicated that both the Richards and Gompertz models successfully described the growth curves of microencapsulated and non-encapsulated free *E. coli* and *Saccharomyces cerevisiae* cultures. The values of asymptote value, *A*, maximum growth rate, μ_m, and lag time, λ, calculated by the modified Gompertz model were very close to that calculated by the modified Richards model. However, the authors preferred the modified Gompertz model because it has three parameters that make it simpler and easier to use in addition to more robust as the parameters are less

Table 2 Growth parameters and their $\alpha = 0.005$ limits, R^2 and *RMSE* of the fit generated by modified Gompertz model for the average OD growth curves of the identified bacterial strains at pH 7.0, 30 ± 1 °C, 110 rpm in MSM supplemented with 1000 mg L^{-1} butyric acid with 1 mL of 2.0, OD_{600} inoculum

Bacterial strain	λ [h]	μ_m [h^{-1}]	a	*m* [h]	*b* [h^{-1}]	R^2	*RMSE*
Alcaligenes sp. SY1	8.47	0.09	1.59 (± 0.13)	14.88 (± 0.76)	0.16 (± 0.03)	0.997	0.003
Pseudomonas aeruginosa	5.72	0.10	1.31 (± 0.11)	10.49 (± 0.72)	0.21 (± 0.05)	0.987	0.005
Achromobacter animicus	5.54	0.08	1.40 (± 0.36)	11.87 (± 0.23)	0.16 (± 0.01)	0.999	0.0002
Achromobacter xylosoxidans	6.71	0.15	1.41 (± 0.15)	10.24 (± 0.53)	0.28 (± 0.08)	0.986	0.007
Serratia marcescens	5.75	0.07	1.55 (± 0.07)	13.54 (± 0.46)	0.23 (± 0.05)	0.999	0.0003
Bacillus methylotrophicus	7.31	0.11	1.34 (± 0.09)	11.61 (± 0.55)	0.43 (± 0.07)	0.982	0.004
Bacillus cereus	5.90	0.09	1.37 (± 0.13)	11.61 (± 0.79)	0.17 (± 0.04)	0.987	0.003
Lysinibacillus fusiformis	5.73	0.10	1.25 (± 0.05)	10.11 (± 0.39)	0.38 (± 0.05)	0.995	0.002
Bacillus subtilis	5.62	0.11	1.06 (± 0.08)	9.32 (± 0.51)	0.27 (± 0.06)	0.992	0.003

correlated. Moreover, the shape parameter in the Richards model is difficult to explain biologically (Bahçeci and Acar 2007). The contrasts in the best fitting model conclusions reached by the authors could be to some extent explained by the use of different microorganisms grown under different environmental conditions as well as the use of different biomass concentrations, measurement methods and the number of experimental data points.

Conclusions

In the present work, 9 out of a total of 24 isolated indigenous bacterial strains that were screened for the capability to utilise butyric acid as a sole source of carbon and energy could be adapted to perform butyric acid degradation. Based on 16S rRNA gene analysis, these strains were identified as *Alcaligenes* sp. strain SY1, *Achromobacter animicus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Achromobacter xylosoxidans*, *Bacillus cereus*, *Lysinibacillus fusiformis*, *Bacillus methylotrophicus* and *Bacillus subtilis*. The bacterial strains were capable of degrading 1000 mg L^{-1} butyric acid within 20 to 24 h at an incubation temperature of 30 ± 1 °C, agitation rate of 110 rpm and pH 7. The growth patterns of the bacterial strains in pure culture utilising butyric acid as the sole source of carbon and energy was well described by the modified Gompertz model. Prediction from primary models such as the modified Gompertz model is a useful tool to predict the behaviour of the bacterial strains isolated in this work in real pit latrine environmental conditions. However, this model has to be investigated under a range of environmental conditions (*inter alia;* temperature, medium and pH) to demonstrate its validity. It is confirmed for the first time that the effectiveness with which the isolated bacterial strains degraded butyric acid has the potential applicability to bioremediation for the control of the odour as a result of butyric acid in the pit latrines. Nevertheless, the mechanisms involved in the degradation of butyric acid for each bacterial strain need to be further investigated. It is likely that the results of this work have provided a basis upon which further investigations of butyric acid-degrading bacteria from pit latrine faecal sludge can be carried out for microbial deodorization of pit latrines stench using in situ microbial community. This could also be used to develop other microbial-based pit latrine faecal sludge deodorization technologies and strategies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Human or animal participants No humans or animals were used in this work.

Informed consent N/A

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