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**The effect of** ***Alcanivorax borkumensis* SK2, a hydrocarbon-metabolising organism, on gas holdup in a 4-phase bubble column bioprocess.**

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* Gas Holdup.
* Bubble Column Reactor.
* Hydrocarbon-based bioprocess.
* *Alcanivorax borkumensis* SK2.
* Multiphase System.

Highlights:

* SK2 biomass was used for the first time to study the hydrodynamics of bubble column reactors.
* Gas holdup was investigated under different operational conditions.
* Gas holdup increased linearly with increasing superficial gas velocity.
* Solid type and loading had significant and differing effects on gas holdup.
* The highest gas holdup occurred in the air-water-SK2 biomass-hydrocarbon system.

Abbreviations:

|  |  |
| --- | --- |
| BCR | Bubble column reactor |
| $$Ɛ\_{G}$$ | Gas holdup |
| Ho | Height of the liquid in the column before aeration  |
| H | Height of the liquid during steady-state aeration  |
| HMO | Hydrocarbon-metabolising organisms |
| *HC* | Hydrocarbon concentration  |
| *MC* | Microbial concentration  |
| STR | Stirred tank reactor |
| *UG* | Superficial gas velocity |

# Abstract

In order to design bioprocesses utilising hydrocarbon-metabolising organisms (HMO) as biocatalysts, the effect of the organism on the hydrodynamics of bubble column reactor (BCR), such as gas holdup, needs to be investigated. Therefore, this study investigates the first use of a HMO, *Alcanivorax borkumensis* SK2, as a solid phase in the operation and hydrodynamics of a BCR. The study investigated the gas holdup in 3-phase and 4-phase systems in a BCR under ranges of: superficial gas velocities (*UG*) from 1 to 3 cm/s, hydrocarbon (chain length C13-21) concentrations (*HC*) of 0, 5, and 10 % v/v and microbial concentrations (*MC*) of 0, 0.35, 0.6 g/l. The results indicated that *UG* was the most significant parameter, as gas holdup increases linearly with increasing *UG* from 1 to 3 cm/s. Furthermore, the addition of hydrocarbonsinto the air- deionized water -SK2 system showed the highest increase in the gas holdup, particularly at high *UG* (above 2 cm/s). The solids (yeast, cornflour, and SK2) phases had differing effects on gas holdup, potentially due to the difference in surface activity. In this work, SK2 addition caused a reduction in the fluid surface tension in the bioprocess which therefore resulted in an increase in the gas holdup in BCR. This work builds upon previous investigations in optimising the hydrodynamics for bubble column hydrocarbon bioprocesses for the application of alkane bioactivation.

# 1 Introduction

Hydrocarbons are abundant, inexpensive, and energy-rich substrates, leading to their use as a low-value fuel and inexpensive carbon feedstock in global industrial markets. Hydrocarbon substrates are relatively unreactive and difficult to functionalise, due to strong C–H bonds [1–4]. Therefore, converting these relatively low-value compounds into higher-value functionalised compounds is a challenge. A potential approach to upgrading these low-value alkane substrates to higher-value products could be through biological oxidation processes, whereby an oxygen moiety is delivered to the alkane’s backbone through the activity of a microorganism [5–7]. These biological systems require an appropriate bioreactor system. Further, because of the relatively underdeveloped nature of this approach, the upgrading bioprocess needs significant development, particularly in finding or modifying a suitable microorganism, as well as optimising the bioreactor system in which this conversion takes place.

One of the key parameters in developing a hydrocarbon upgrading bioprocess is the choice of microorganism, which should have the ability to add an oxygen group into the alkanes (commonly using cytochrome P450 enzymes). In nature, a range of organisms, including bacterial and fungal species, are able to metabolise hydrocarbon substrates in the environment and industrial biotechnology [6,8–14]. Of these organisms *Alcanivorax* species have been shown to play a major role in degrading hydrocarbons, predominantly alkanes and alkenes, in the environment [12,13,15–21], including after major disasters like the Deepwater Horizon oil spill [22]. This makes it an attractive organism for hydrocarbon-based bioprocesses and bioremediation of hydrocarbon pollutants if its hydrocarbon metabolism can be redirected toward the production of useful compounds [23–25].

Another key parameter in such aerobic bioprocesses is the bioreactor system, which hosts the multiphase process and covers (in particular) the oxygen demand of the organisms’ metabolism. One of the most promising bioreactors in aerobic bioprocess applications is the aerated bubble column reactor (BCR). BCRs have been widely utilised in numerous laboratory and industrial applications such as chemical, petrochemical, and biochemical processes, water treatment, and separation processes, as well as fermentation processes [26–32]. Moreover, BCRs also provide sufficient mass transfer, as well as low shear damage, especially for sensitive cells [33,34]. Despite their obvious utility in these sorts of systems, BCR systems demonstrate complex hydrodynamics, particularly when the process contains non-miscible phases such as hydrocarbons, aqueous- and solid phases. Therefore, improved understanding of system dynamics is needed when utilising BCRs, particularly in multi-phase systems and more particularly when using poorly understood microbes, such as *Alcanivorax borkumensis* SK2 (Hereafter referred to simply by its strain designation, SK2).

In previous work, the impact of operational conditions such as superficial gas velocity, hydrocarbon concentration, solids loading, and solids type have been investigated in hydrocarbon-based bioprocesses in BCRs. In particular, the effect on the liquid-liquid (hydrocarbon-aqueous) mixing behaviour [35], gas holdup, bubble size [36], gas-liquid interfacial area [37], and overall oxygen transfer [38], have been studied. However, very little work has been performed on utilising hydrocarbon-degrading bacteria in hydrocarbon-based bioprocesses. The effect of these organisms in BCRs, particularly on the hydrodynamics such as gas holdup, is not yet understood. In order to design bioprocesses utilising hydrocarbon-degrading organisms, the effect of the organisms and their by-products needs to be investigated.

In this study, in order to elucidate how SK2 biomass might be grown optimally before operating as a biological catalyst, we examined the growth of SK2 biomass using sodium pyruvate, *n-*octane, and *n-*hexadecane as carbon sources, and under cultivation temperatures of 25 oC, 30 oC, and 37 oC. Moving into a pilot-scale BCR, the effect of adding SK2 biomass and modifying operational conditions such as superficial gas velocity and hydrocarbon (long-chain C13-C21 mixture) concentration on gas holdup was studied. The outcomes of this experimental investigation ultimately provide a fundamental understanding of the hydrodynamics of aerated BCRs, with the gas holdup as a critical parameter in designing and operating the bioreactor system. Moreover, this study, for the first time, provides information on utilising hydrocarbon-degrading bacteria in bioreactor systems like BCRs, for the upgrading of hydrocarbons to more valuable compounds.

# 2 Materials and Methods

## 2.1 Growth of *Alcanivorax borkumensis* SK2

A mixture of *n-*alkanes (chain length C13-21) supplied by Sasol Wax (via Organic Synthesis), South Africa was used in this work. This mixture was analysed via gas chromatography (6890 N, Agilent technologies network) and the hydrocarbon mixture was composed of, per volume, 0.81 % *n*-C13, 28.06 % *n*-C14, 26.62 % *n*-C15, 22.67 % *n*-C16, 15.51 % *n*-C17, 5.37 % *n*-C18, 0.82 % *n*-C19, 0.10 % *n*-C20 and 0.03 % *n*-C21, as reported previously [35–38]. This study utilised deionized water, air, and the hydrocarbon-metabolising organism *Alcanivorax* *borkumensis* SK2.

*Alcanivorax* *borkumensis* SK2 was obtained from the NCIMB culture collection (Aberdeen, UK). SK2 was routinely cultured on YTSS medium agar plates with 1 % sodium pyruvate (w/v) as the carbon source. SK2was incubated in YTSS liquid medium, which contained (per 1000 ml deionised water): yeast extract (4 g), tryptone (2.5 g), sea salts (20 g) (Sigma)[containing 2×104 mg/l chloride, 1.1×104 mg/l sodium, 2660 mg/l sulfate, 350 mg/l potassium, 400 mg/l calcium, 170 mg/l carbonates, 5.6 mg/l boron, 1320 mg/l magnesium, and 8.8 mg/l strontium], agar (20 g) [17,39]. All YTSS reagents were supplied by Sigma Aldrich. Different carbon sources, including *n-*alkanes (e.g., 0.5 %v/v *n-*octane and *n-*hexadecane) and sodium pyruvate (10 g/l) were used as the sole carbon source for bacterial growth. Once all the required YTSS components and 1 % sodium pyruvate had been added (when appropriate for the experiment), the baffled flasks were autoclaved with cotton and aluminum foil at 121 oC for 20 minutes for sterilisation. *n-*alkanes were filter-sterilized using a 0.2 µm filter and then axenically added to the YTSS baffled flasks after sterilisation, as needed for each experiment.

Cultures to be used as inoculum were grown at 30 oC in baffled 100 ml flasks with 50 ml YTSS supplemented with 1 % pyruvate, in a shaker incubator at 150 rpm. Cultures in the late exponential phase were used to inoculate growth experiments. Thereafter, baffled 100 ml flasks with 50 ml YTSS broth were used for growth experiments. Subsequently, each sterilised YTSS flask was inoculated with 250 µl starter culture of SK2 aseptically in the laminar flow cabinet, giving an initial OD600nm of approximately 0.1. The baffled flasks were then placed in shaking incubators at 150 rpm, and with the following incubation temperatures: 25 oC, 30 oC, and 37 oC. To measure growth, optical density measurements (at a wavelength of 600 nm) were taken every 24 hours of the aqueous phase using a Jenway 6305 Spectrophotometer.

## 2.2 Bioreactor operation experiments

Figure 1 illustrates the experimental setup, which was comprised of a laboratory-scale bubble column reactor (with an inner diameter of 15 cm and a height of 90 cm) made of tubular glass (Glasstech, South Africa). The column reactor was filled with the experimental liquid up to 26 cm above the gas sparger, giving a total working volume of 4.6 liters. Air bubbles were provided by air sparging through a porous (made of carborundum with a pore size of approximately 40 μm) air stone with a 14 cm diameter at the base of the column. The superficial gas velocity was maintained using a high-flow rotameter (Cole-Parmer 150 mm).

At the top of the column, an air outlet made provision for the escape of undissolved air. The air outlet was then connected to a condenser that condensed and trapped the alkanes to prevent them from exiting into the laboratory environment, as well as to keep the system composition constant. An outlet was provided at the reactor bottom for the discharge of the slurry after each experiment. The glass column was placed inside a bespoke rectangular Perspex box which was filled with temperature-controlled tap water to maintain the temperature of the system at 23 oC ± 2 oC.

The gas holdup was analysed by visual observation with gradations on the reactor wall and then calculated as the fraction of gas in the bubble column system using Equation 1 [40,41]. Ho is the liquid height in the column before aeration and H the liquid height in the column after aeration. Gas holdup readings were taken after ten minutes (estimated as a steady state) by the ruler attached to the top zone of the BCR.

$Ɛ\_{G}=\frac{H-H\_{0}}{H}$ Equation 1

In order to collect biomass for bioreactor experiments with varying biomass concentrations, cultures of SK2 were cultivated in YTSS media with sodium pyruvate, as described in 2.1. After a cultivation time of 14 days, the biomass was collected and transferred to 50 mL Eppendorf centrifuge tubes and centrifuged (Eppendorf 5702 R) at 4,400 × g for 10 minutes. The growth medium was removed from the Eppendorf tubes using a Pasteur pipette, leaving only the biomass in the tubes. The Eppendorf tubes were filled again with sterilised water and vortexed to resuspend the cells. The tubes were centrifuged again under the same conditions. This washing procedure was repeated three times to reduce any extra-cellular compounds in the process and therefore prevent interference in BCRs. Once biomass had been prepared, the appropriate amount was added to BCRs, along with water and hydrocarbons, as the experiment required.

A biomass sample was taken from a culture in the stationary phase, and several dilutions were made to measure optical density, as well as the dry cell weight using 0.2 μm filters (Millipore). Thereafter, the optical density versus dry cell weight standard curve was generated to measure the concentration of the biomass in each flask before being added to the column.

The experiments were performed in air-water, air-water-SK2 biomass, and air-water-SK2 biomass-hydrocarbons in the bubble column under a range of various operating conditions such as superficial gas velocity (0.5 cm/s to 3 cm/s), hydrocarbon concentration (0 % v/v, 5 % v/v, and 10 % v/v), and microbial solids concentration (0 g/l, 0.35 g/l, and 0.6 g/l) in a bubble column. The bubble column (with 15 cm diameter and 90 cm height) utilised in this work is practically considered a large column at the laboratory scale. Therefore, providing enough microbial concentration might be very challenging for such a bioprocess. However, the SK2 biomass (0.6 g/l) in this study was comparable to the minimum solid (deactivated yeast and cornflour) concentrations used in previous studies and provides a solid starting point for further studies investigating biomass concentration effects. The effect of SK2 biomass on fluid surface tension (in the air-water-SK2 biomass system) was measured by Force Tensiometer – Sigma 702. The surface tension measurements were taken for separate phases due to the complexity of the immiscible phases (air-water-hydrocarbon-SK2) mixture which ultimately results in separation in the measuring vessel.

The experimental conditions, however, were selected at the mentioned ranges for two reasons; firstly, to be comparable to the previous findings in the same system, and secondly, to avoid the accumulation of foam in the bioreactor system, especially at high aeration rates. The foaming phenomena is a significant challenge in the bioprocess, particularly in this process where multiple phases (e.g., air, water, hydrocarbons, and SK2 biomass) were brought together in a reactor system. This practical work demonstrates the concept of laboratory investigation of real bioprocess, which would assist in understanding the industrial applications where mixtures of multi-phase substances are used.



Figure 1. Diagram of the experimental setup of the bubble column hydrocarbon-based bioprocess.

# 3 Results and Discussion

## 3.1 Effect of carbon source and incubation temperature on the growth of SK2

In order to test the effect of the SK2 biomass on the operation of the reactor, in the first instance sufficient biomass was needed. To identify the maximal growth conditions, a range of growth temperatures and carbon sources for SK2 were investigated. The carbon sources were chosen based on the expectation that SK2 would be able to utilise both linear alkanes (e.g. *n-*octane, *n-*hexadecane) or sodium pyruvate [18]. To evaluate which carbon source and temperature would result in maximal growth, SK2 was cultivated at small volume (50 mL), in triplicate, at three different temperatures (25 oC, 30 oC, 37 oC) in YTSS medium supplemented with one of three carbon sources: *n-*octane, *n-*hexadecane or sodium pyruvate. The results of this experiment are presented in Figure 2.

SK2 was able to utilise all three of these carbon sources, with a shifting preference depending on the temperature. At lower temperatures (25 oC and 30 oC), the growth rates were significantly faster when SK2 was cultured on hydrocarbons than on sodium pyruvate. However, a significant lag phase was observed when cells were cultivated on *n-*octane. At 37 oC, all three substrates performed similarly. Previously, maximal growth rates of SK2 have been reported when cells were cultured at 30 oC with *n-*alkanes ranging from C14-19 [18]. However, we observed similar growth at 30 oC when cells were cultured on *n-*octane or *n-*hexadecane. These results suggest that temperature may have a major effect on either the uptake or metabolism of *n-*alkanes of different lengths, which should be taken into account when utilising these substrates as a carbon source for SK2. Overall, *n*-alkanes (*n-*octane or *n-*hexadecane) substrates can be used to produce a satisfactory growth of SK2 biomass at cultivation temperatures of 25 oC and 30 oC. For gas holdup experiments, it was required to grow SK2 in a non-alkane substrate (sodium pyruvate is the only other carbon source on which SK2 is known to grow on other than hydrocarbons) at a cultivation temperature of 37 oC before being added to the bubble column, containing hydrocarbons.

**Time (h)**

**Time (h)**

**Time (h)**

**37 oC**

**30 oC**

**25 oC**

Figure 2: Growth of SK2 at different incubation temperatures (25 oC, 30 oC, and 37 oC) and with different carbon sources (sodium pyruvate, n-octane, and n-hexadecane). Error bars represent the standard deviation of three biological replicates.

## 3.2 Effect of three-phase systems on gas holdup

The effect of superficial gas velocity and SK2 biomass concentration on gas holdup in three-phase (air-water-SK2 biomass) systems were then evaluated (Figure 3). Specifically, we quantified the variation of gas holdup under superficial gas velocities of 0.5 cm/s to 3 cm/s at different concentrations of SK2 biomass (0 g/l, 0.35 g/l, and 0.6 g/l). Gas holdup increased with the addition of SK2 biomass into the bioreactor system, except at the lowest superficial gas velocity of 0.5 cm/s. Gas holdup also increased almost linearly with an increasing superficial gas velocity at all SK2 biomass concentrations. In two-phase systems (0 g/l solids), the value of gas holdup increased from 0.05 m/m to 0.17 m/m as the superficial gas velocity was increased from 0.5 cm/s to 3 cm/s. Similar trends were observed with increased solid concentrations (0.35 g/l and 0.6 g/l) at constant superficial gas velocity. These findings confirm that the bubble column system was operating in a homogenous flow regime, as reported by Sharaf and co-workers [42].

One potential source of this effect is the presence of solids in suspension in the reactor (in this case, the SK2 biomass). The SK2 biomass concentrations used in this study (0.35 g/l and 0.6 g/l) were chosen to be comparable to the concentrations of other solids used in previous studies (deactivated yeast and cornflour) [36,37], and this comparison provides a solid starting point for further studies investigating biomass concentration effects, particularly at higher biomass concentrations.

The effects of particle size and concentration on gas holdup have been widely studied by numerous research groups [43–45] who report that the increase in either solid (glass beads and iron oxides catalyst) size or concentration results in a notable reduction of the gas holdup values in the system. Clarke and colleagues studied the hydrodynamics of hydrocarbon-based bioprocess in bubble columns [35–38] and stirred tank reactors [7,46–49] with deactivated S. cerevisiae or cornflour as solids. Comparing the observations found in this study with the previous work in Figure 4, a decrease in gas holdup is seen with the addition of deactivated yeast, while no change is found with the addition of cornflour. This solid effect on gas holdup is unlikely to be related to the size of the solids (the size trend does not predict the effect on gas holdup) but it is attributed to the effect of the solids on fluid properties, as stated in Table 1.

These findings are more likely to be related to the surface effects of the solids themselves which might affect the fluid properties, e.g., viscosity and surface tension, and thereafter result in differing behaviors of gas holdup in the systems. The addition of yeast was found to reduce the surface tension, and slightly increase the viscosity in the previous study [35]. Whereas the addition of cornflour resulted in a small increase in the viscosity and an insignificant change in the surface tension. The fluid properties, however, were significantly affected by the addition of SK2, where a reduction of surface tension was observed with an increase in the fluid viscosity (Table 1). Other studies [50–56] have found that any reduction in surface tension ultimately decreases the size of gas bubbles in the reactor system, increasing the gas holdup thereafter. In other words, small-sized bubbles generally have lower rise velocities than big bubbles, which would likely allow the small bubbles to remain longer in the reactor, and therefore enhance the value of gas holdups [57–59]. A higher value of the gas holdup would eventually lead to a significant increase in the gas-liquid area, as well as enhance the overall oxygen transfer in the bioprocess [37,46,60,61].



Figure 3: Gas holdup measurements under a range of superficial gas velocity and microbial concentrations (0 g/l, 0.35 g/l, and 0.6 g/l) in two-phase (air-water) and three-phase (air-water-SK2 biomass) systems in the BCR. Error bars represent the standard deviation of three readings which are smaller than the data points.



Figure 4: Comparison of the gas holdup of different solid types (e.g., yeast, cornflour, SK2 biomass) and solids loading 0 g/l and 0.6 g/l under a variation for superficial gas velocities (0.5 to 3 cm/s) in BCR. Error bars representing the standard deviation of three readings are smaller than the data points.

Table 1: Results of the fluid viscosity and surface tension using a Physica MCR 501 and Sigma 702 respectively at 25 oC and 1 atm.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Phase | Solid loading (g/l) | Average particle size (µm) | Viscosity (mPa*•*s) | Surface tension (mN/m) |
| Water | / | / | 0.86 | 72.86 |
| Water + yeast | 0.6 | 5 | 0.99 | 46.54 |
| Water + cornflour | 0.6 | 13.36 | 1.00 | 70.33 |
| Water + SK2 | 0.6 | 7.5 | 1.49 | 55.67 |

## 3.3 Effect of four-phase systems on gas holdup.

In order to examine the effect of the solids phase on hydrocarbon bioprocesses, rather than only 3-phase systems, hydrocarbons as a fourth phase were introduced. Experiments were performed under a range of operating conditions including: superficial gas velocity (0.5 cm/s to 3 cm/s), hydrocarbon concentration (0 % v/v, 5 % v/v, and 10 % v/v), while maintaining a constant SK2 concentration of 0.6 g/l (Figure 5). Gas holdup increased with increases in superficial gas velocity (from 1 cm/s to 3 cm/s) as well as with increasing hydrocarbon concentration (from 0 to 10 % v/v). This is in agreement with other literature studies [29,62–66]. The impact of hydrocarbon addition was recently investigated in the 3-phase (air-water-hydrocarbon) system, with increases in hydrocarbon concentration resulting in a slight decrease in gas holdup values [36]. This reduction in gas holdup was a result of increasing the overall liquid viscosity, as the concentration of hydrocarbons increased in the system [53]. Nevertheless, in a 4-phase (air-water-hydrocarbon-yeast) bubble column [36], it was reported that changes in hydrocarbon concentration (from 2.5 % v/v to 20 % v/v), while maintaining a constant concentration of yeast (*Saccharomyces cerevisiae*), had an insignificant effect on gas holdup, or on the system hydrodynamics.

It is clearly observed that the interaction between SK2 biomass and hydrocarbons in this experimental study resulted in different trends of gas holdup from previous work where deactivated yeast and cornflour were used as solids phases [36,37]. One previous study which tested the effect of hydrocarbons on fluid properties such as surface tension and viscosity found that increases in hydrocarbon concentration resulted in an increase in the fluid viscosity (from 0.86 to 2.2 mPa*•*s) and a decrease in the surface tension (from 72.86 to 26.31 mN*/*m) [35]. Other studies supported these findings [7,46,67,68]. For example, Rols and colleagues [69] demonstrated that hydrocarbons perform as surface-active agents in air-water-hydrocarbon systems, which reduces the surface tension of the fluid, as well as decreases the size of bubbles in the system. Furthermore, the addition of hydrocarbons to the 3-phase (air-water-SK2 biomass) system resulted in a non-homogeneous mixture, with the hydrocarbons largely remaining on the top zone of the aqueous phase, particularly at low superficial gas velocities (<1 cm/s). Consequently, good mixing of the 4-phase system was experimentally established at a 1 cm/s aeration rate and above [35].

On the other hand, the presence of hydrocarbon-degrading organisms (such as SK2) in hydrocarbon-contaminated environments is widely reported [12,17,18,70–72]. Many of these species, including SK2, produce biosurfactants such as glucolipids, during the degradation process, which increases the bioavailability of hydrocarbons as an energy and carbon source [70,73,74]. The observed influence of SK2 biomass on gas holdup was separated from biosurfactant concentration as much as possible by using several biomass-washing steps, to remove exogenous compounds. Therefore, the effect on gas holdup is likely due to the surface activity (the ability of cells in reducing the surface tension and undergo surface cell rearrangement during the cultivation process) of SK2 itself. Concerning the effect of biosurfactants on this bioprocess, the fluid surface tension dropped (reported in Table 1) as SK2 increased in the system which likely resulted in an increase in the number of small bubbles which eventually enhanced the value of gas holdup. Therefore, the presence of a surface active solid in the bioprocess helps to reduce the bubble coalescence phenomena, and thus, the system operates in a homogenous-bubbly flow regime [75,76]. Furthermore, previous studies have shown that when the experimental liquid contains any amount of surfactants (synthetic surfactant or/and biosurfactant), the amphiphilic molecules of these surfactants would possibly accrue on the surface of the bubble, reducing its rise velocity in the reactor system, and thereafter increase the gas holdup [77–79]. A study by Li and colleagues [55] reported that gas holdup increased with increasing surfactant (sodium dodecyl sulfate) concentrations in a BCR. The effect of biosurfactants on the process is an important process consideration, which requires further investigation.



Figure 5: Gas holdup measurements under a range of superficial gas velocities, and hydrocarbon concentrations (0 %v/v, 5 %v/v, and 10 %v/v) at constant solid loadings (0.6 g/l) in 4-phase (air-water- SK2 biomass-hydrocarbon) system in a BCR. Error bars representing the standard deviation across three readings are smaller than the data points.

To clarify whether the accumulation of biosurfactants in the bioprocess affects gas holdup, and the system in general, we examined the gas holdup in two-phase and four-phase systems over a period of time, allowing the organism to produce biosurfactants in the system. Figure 6 illustrates the gas holdup measurements for air-water and air-water-SK2 biomass-hydrocarbon systems at constant superficial gas velocity (midpoint 2 cm/s). Unsurprisingly, it was observed that gas holdup did not change in the air-water system over a duration of 4 days, whereas, in the four-phase system, the gas holdup increased significantly over the same period. The recorded gas holdup on day 4 was the highest value recorded in this study and exceeded values recorded in previous gas holdup investigations [36,37] which likely indicates the presence of biosurfactants in the bioprocess, especially when the process contains hydrocarbon-degrading bacteria as a solid phase.

 

Figure 6: Measurements of gas holdup over time at a constant superficial gas velocity (2 cm/s) for 2-phase (air-water), and 4-phase (air-water-SK2 biomass (0.6 g/l)-hydrocarbon 10 % v/v) systems in BCRs. Error bars represent the standard deviation across three readings which are smaller than the data points.

# 4 Conclusions

In this work, the growth conditions of SK2 were experimentally investigated with different carbon sources (i.e., sodium pyruvate, *n-*octane, and *n-*hexadecane) under a range of cultivation temperatures, with hydrocarbons shown to be the preferred carbon source at 25 oC and 30 oC. It was found that gas holdup values were significantly affected by all operational inputs (*UG*, *HC*, and *MC*), with the highest value of gas holdup found in a 4-phase system. An increase in *UG* from 1 cm/s to 3 cm/s resulted in a linear increase in a gas holdup in BCR. It was also found that the addition of hydrocarbons with the presence of SK2 biomass into the reactor significantly increased the gas holdup. These findings suggest that the addition of SK2 resulted in a significant reduction in surface tension which ultimately increase the gas holdup. This work is the first analysis of the use of hydrocarbon-degrading organisms in a BCR operation and shows that the solid phase has a significant impact on the fluid properties and gas holdup. The work represents a move towards developing a commercial bioprocess for hydrocarbon activation, utilising hydrocarbon-degrading organisms which are naturally able to metabolise these materials and potentially produce higher-value compounds.

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