-

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

1,2Ayman A. Abufalgha, 2Andrew R. J. Curson, 2David J. Lea-Smith, and 1Robert W. M. Pott

DST-NRF Centre of Excellence in Catalysis (c\* change), South Africa

1Department of Process Engineering, Stellenbosch University, Stellenbosch 7600, South Africa.

2School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, United Kingdom.

**1Corresponding Author:** A/Prof. R.W.M. Pott

Department of Process Engineering, Banghoek Road, Stellenbosch 7600, South Africa.

E-mail address: [rpott@sun.ac.za](mailto:rpott@sun.ac.za)

Keywords:

* 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 |
|  | 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.

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.

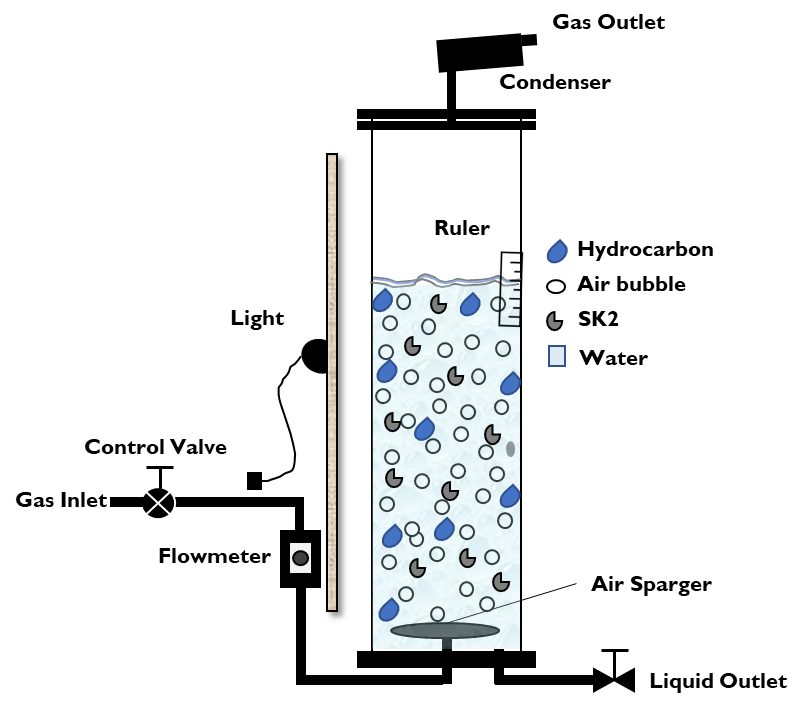


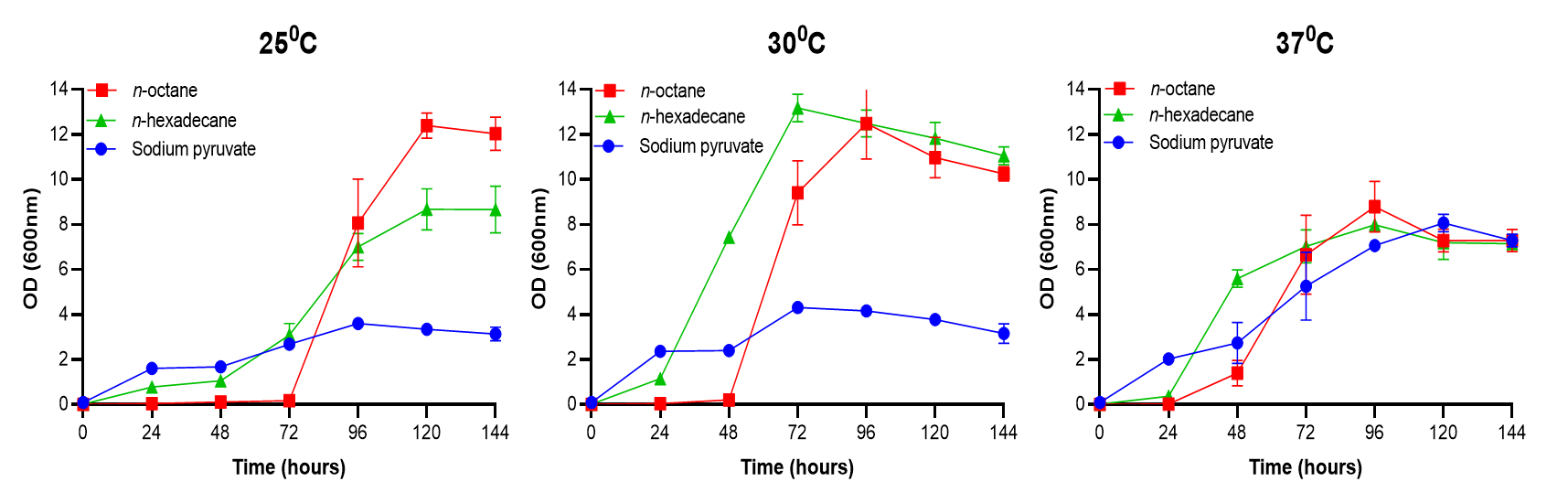
Figure 1. Diagram of the experimental setup of the bubble column hydrocarbon-based bioprocess.

# 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.

# 5 Acknowledgements

Part of this work was performed in the School of Biological Sciences, University of East Anglia, the UK, and was supported by a University of East Anglia Global Challenges Research Fellowship. The remaining work was performed in the Department of Process Engineering, University of Stellenbosch, South Africa, and was financially supported by the Centre of Excellent in Catalysis (c\*change), and Stellenbosch University. A. Curson and D. Lea-Smith acknowledge support from Human Frontier Science Program grant RGP0031.

# 6 References

[1] O. Grundmann, A. Behrends, R. Rabus, J. Amann, T. Halder, J. Heider, F. Widdel, Genes encoding the candidate enzyme for anaerobic activation of n-alkanes in the denitrifying bacterium, strain HxN1, Environmental Microbiology. 10 (2008) 376–385. https://doi.org/10.1111/j.1462-2920.2007.01458.x.

[2] P. Gandeepan, L. Ackermann, Transient Directing Groups for Transformative C–H Activation by Synergistic Metal Catalysis, Chem. 4 (2018) 199–222. https://doi.org/10.1016/j.chempr.2017.11.002.

[3] F. Roudesly, J. Oble, G. Poli, Metal-catalyzed C[sbnd]H activation/functionalization: The fundamentals, Journal of Molecular Catalysis A: Chemical. 426 (2017) 275–296. https://doi.org/10.1016/j.molcata.2016.06.020.

[4] S.J. Freakley, S. Kochius, J. van Marwijk, C. Fenner, R.J. Lewis, K. Baldenius, S.S. Marais, D.J. Opperman, S.T.L. Harrison, M. Alcalde, M.S. Smit, G.J. Hutchings, A chemo-enzymatic oxidation cascade to activate C–H bonds with in situ generated H2O2, Nature Communications. 10 (2019). https://doi.org/10.1038/s41467-019-12120-w.

[5] J.. Rols, J.. Condoret, C. Fonade, G. Goma, Mecanism of enhanced oxygen transfer in fermentation using emulsified oxygen-vectors, Biotechnology and Bioengineering. 35 (1990) 427–435.

[6] A. Wentzel, T.E. Ellingsen, H.K. Kotlar, S.B. Zotchev, M. Throne-Holst, Bacterial metabolism of long-chain n-alkanes, Applied Microbiology and Biotechnology. 76 (2007) 1209–1221. https://doi.org/10.1007/s00253-007-1119-1.

[7] K.G. Clarke, L.D.C. Correia, Oxygen transfer in hydrocarbon–aqueous dispersions and its applicability to alkane bioprocesses: A review, Biochemical Engineering Journal. 39 (2008) 405–429. https://doi.org/10.1016/j.bej.2007.11.020.

[8] J. Shennan, J.. Levi, The growth of yeasts on hydrocarbons, Progress in Industrial Microbiology. 13 (1974) 1–57.

[9] T.H.M. Smits, B. Witholt, J.B. van Beilen, Functional characterization of genes involved in alkane oxidation by Pseudomanas aeruginosa, Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology. 84 (2003) 193–200. https://doi.org/10.1023/A:1026000622765.

[10] F. Rojo, Degradation of alkanes by bacteria: Minireview, Environmental Microbiology. 11 (2009) 2477–2490. https://doi.org/10.1111/j.1462-2920.2009.01948.x.

[11] C.W. Greer, J.B. van Beilen, D. Labbe, T.H.M. Smits, L.G. Whyte, B. Witholt, Gene Cloning and Characterization of Multiple Alkane Hydroxylase Systems in Rhodococcus Strains Q15 and NRRL B-16531, Applied and Environmental Microbiology. 68 (2002) 5933–5942. https://doi.org/10.1128/aem.68.12.5933-5942.2002.

[12] H. Akihiro, S. Kazuaki, H. Shigeaki, Alcanivorax which prevails in oil‐contaminated seawater exhibits broad substrate specificity for alkane degradation, Environmental Microbiology. 5 (2003) 746–753. https://doi.org/10.1046/j.1468-2920.2003.00468.x.

[13] W. Wang, L. Wang, Z. Shao, Diversity and Abundance of Oil-Degrading Bacteria and Alkane Hydroxylase (alkB) Genes in the Subtropical Seawater of Xiamen Island, Microbial Ecology. 60 (2010) 429–439. https://doi.org/10.1007/s00248-010-9724-4.

[14] I.M. Head, D.M. Jones, W.F.M. Röling, Marine microorganisms make a meal of oil., Nature Reviews. Microbiology. 4 (2006) 173–182. https://doi.org/10.1038/nrmicro1348.

[15] Y. Kasai, H. Kishira, T. Sasaki, K. Syutsubo, K. Watanabe, S. Harayama, Predominant growth of, 4 (2002) 141–147.

[16] F. Coulon, B.A. McKew, A.M. Osborn, T.J. McGenity, K.N. Timmis, Effects of temperature and biostimulation on oil-degrading microbial communities in temperate estuarine waters, Environmental Microbiology. 9 (2007) 177–186. https://doi.org/10.1111/j.1462-2920.2006.01126.x.

[17] M.M. Yakimov, P.N. Golyshin, S. Lang, E.R.B. Moore, W.R. Abraham, H. Lünsdorf, K.N. Timmis, Alcanivorax borkumensis gen. nov., sp. nov., a new, hydrocarbon- degrading and surfactant-producing marine bacterium, International Journal of Systematic Bacteriology. 48 (1998) 339–348. https://doi.org/10.1099/00207713-48-2-339.

[18] D.J. Naether, S. Slawtschew, S. Stasik, M. Engel, M. Olzog, L.Y. Wick, K.N. Timmis, H.J. Heipieper, Adaptation of the hydrocarbonoclastic bacterium Alcanivorax borkumensis SK2 to alkanes and toxic organic compounds: A physiological and transcriptomic approach, Applied and Environmental Microbiology. 79 (2013) 4282–4293. https://doi.org/10.1128/AEM.00694-13.

[19] C. Liu, Z. Shao, Alcanivorax dieselolei sp. nov., a novel alkane-degrading bacterium isolated from sea water and deep-sea sediment, International Journal of Systematic and Evolutionary Microbiology. 55 (2005) 1181–1186. https://doi.org/10.1099/ijs.0.63443-0.

[20] E. Manilla-Pérez, A.B. Lange, S. Hetzler, M. Wältermann, R. Kalscheuer, A. Steinbüchel, Isolation and characterization of a mutant of the marine bacterium alcanivorax borkumensis sk2 defective in lipid biosynthesis, Applied and Environmental Microbiology. 76 (2010) 2884–2894. https://doi.org/10.1128/AEM.02832-09.

[21] J. Liu, Y. Zheng, H. Lin, X. Wang, M. Li, Y. Liu, M. Yu, M. Zhao, N. Pedentchouk, D.J. Lea-Smith, J.D. Todd, C.R. Magill, W.J. Zhang, S. Zhou, D. Song, H. Zhong, Y. Xin, M. Yu, J. Tian, X.H. Zhang, Proliferation of hydrocarbon-degrading microbes at the bottom of the Mariana Trench, Microbiome. 7 (2019). https://doi.org/10.1186/s40168-019-0652-3.

[22] J.E. Kostka, O. Prakash, W.A. Overholt, S.J. Green, G. Freyer, A. Canion, J. Delgardio, N. Norton, T.C. Hazen, M. Huettel, Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the deepwater horizon oil spill, Applied and Environmental Microbiology. 77 (2011) 7962–7974. https://doi.org/10.1128/AEM.05402-11.

[23] M. Ayala, E. Torres, Enzymatic activation of alkanes: Constraints and prospective, Applied Catalysis A: General. 272 (2004) 1–13. https://doi.org/10.1016/j.apcata.2004.05.046.

[24] L. Wang, W. Wang, Q. Lai, Z. Shao, Gene diversity of CYP153A and AlkB alkane hydroxylases in oil-degrading bacteria isolated from the Atlantic Ocean, Environmental Microbiology. 12 (2010) 1230–1242. https://doi.org/10.1111/j.1462-2920.2010.02165.x.

[25] S. Kochius, J. van Marwijk, A.C. Ebrecht, D.J. Opperman, M.S. Smit, Deconstruction of the CYP153a6 alkane hydroxylase system: Limitations and optimization of in vitro alkane hydroxylation, Catalysts. 8 (2018). https://doi.org/10.3390/catal8110531.

[26] Y. Kawase, B. Halard, M. Moo-Young, Theoretical prediction of volumetric mass transfer coefficients in bubble columns for Newtonian and non-Newtonian fluids, Chemical Engineering Science. 42 (1987) 1609–1617. https://doi.org/10.1016/0009-2509(87)80165-3.

[27] N. Kantarci, F. Borak, K.O. Ulgen, Bubble column reactors, Process Biochemistry. 40 (2005) 2263–2283. https://doi.org/10.1016/j.procbio.2004.10.004.

[28] P. Rollbusch, M. Bothe, M. Becker, M. Ludwig, M. Grünewald, M. Schlüter, R. Franke, Bubble columns operated under industrially relevant conditions – Current understanding of design parameters, Chemical Engineering Science. 126 (2015) 660–678. https://doi.org/10.1016/J.CES.2014.11.061.

[29] G. Besagni, The effect of operating and design parameter on bubble column performance: The LOPROX case study, Chinese Journal of Chemical Engineering. 40 (2021) 48–52. https://doi.org/10.1016/j.cjche.2020.12.029.

[30] G. Besagni, L. Gallazzini, F. Inzoli, On the scale-up criteria for bubble columns, Petroleum. 5 (2017) 1–9. https://doi.org/10.1016/j.petlm.2017.12.005.

[31] R. Pishgar, A. Kanda, G.R. Gress, H. Gong, J.A. Dominic, J.H. Tay, Effect of aeration pattern and gas distribution during scale-up of bubble column reactor for aerobic granulation, Journal of Environmental Chemical Engineering. 6 (2018) 6431–6443. https://doi.org/10.1016/j.jece.2018.10.006.

[32] F. Ghoddosi, H. Golzar, F. Yazdian, K. Khosravi-Darani, E. Vasheghani-Farahani, Effect of carbon sources for PHB production in bubble column bioreactor: Emphasis on improvement of methane uptake, Journal of Environmental Chemical Engineering. 7 (2019) 102978. https://doi.org/10.1016/j.jece.2019.102978.

[33] J.H. Yoon, J.H. Shin, T.H. Park, Characterization of factors influencing the growth of Anabaena variabilis in a bubble column reactor, Bioresource Technology. 99 (2008) 1204–1210. https://doi.org/10.1016/j.biortech.2007.02.012.

[34] K. Kumar, D. Das, Growth characteristics of Chlorella sorokiniana in airlift and bubble column photobioreactors, Bioresource Technology. 116 (2012) 307–313. https://doi.org/10.1016/j.biortech.2012.03.074.

[35] A.A. Abufalgha, K.G. Clarke, R.W.M. Pott, The liquid-liquid homogeneity of a four phase simulated hydrocarbon-based bioprocess in a bubble column reactor, Journal of Chemical Technology & Biotechnology. (2019). https://doi.org/10.1002/jctb.5989.

[36] A.A. Abufalgha, K.G. Clarke, R.W.M. Pott, Characterisation of bubble diameter and gas hold-up in simulated hydrocarbon-based bioprocesses in a bubble column reactor, Biochemical Engineering Journal. 158 (2020) 107577. https://doi.org/10.1016/j.bej.2020.107577.

[37] A.A. Abufalgha, R.W.M. Pott, J.C. Cloete, K.G. Clarke, Gas–liquid interfacial area and its influence on oxygen transfer coefficients in a simulated hydrocarbon bioprocess in a bubble column reactor, Journal of Chemical Technology and Biotechnology. (2020). https://doi.org/10.1002/jctb.6625.

[38] A.A. Abufalgha, R.W.M. Pott, K.G. Clarke, Quantification of oxygen transfer coefficients in simulated hydrocarbon-based bioprocesses in a bubble column bioreactor, Bioprocess and Biosystems Engineering. 44 (2021) 1913–1921. https://doi.org/10.1007/s00449-021-02571-1.

[39] R. Denaro, L. Giuliano, M.M. Yakimov, M. Genovese, S. Cappello, Predominant growth of Alcanivorax during experiments on “oil spill bioremediation” in mesocosms, Microbiological Research. 162 (2006) 185–190. https://doi.org/10.1016/j.micres.2006.05.010.

[40] S. Schugerl, Oxygen transfer into highly viscous media., Verfahrenstechnik. 4 (1980) 727–730. http://www.scopus.com/scopus/inward/record.url?eid=2-s2.0-0018970646&partnerID=40&rel=R8.0.0.

[41] S. Dhanasekaran, T. Karunanithi, Improved gas holdup in novel bubble column, Canadian Journal of Chemical Engineering. 90 (2012) 126–136. https://doi.org/10.1002/cjce.20509.

[42] S. Sharaf, M. Zednikova, M.C. Ruzicka, B.J. Azzopardi, Global and local hydrodynamics of bubble columns - Effect of gas distributor, Chemical Engineering Journal. 288 (2016) 489–504. https://doi.org/10.1016/j.cej.2015.11.106.

[43] A.K. Jhawar, A. Prakash, Influence of bubble column diameter on local heat transfer and related hydrodynamics, Chemical Engineering Research and Design. 89 (2011) 1996–2002. https://doi.org/10.1016/j.cherd.2010.11.019.

[44] H. Li, A. Prakash, A. Margaritis, M.A. Bergougnou, Effects of micron-sized particles on hydrodynamics and local heat transfer in a slurry bubble column, Powder Technology. 133 (2003) 171–184. https://doi.org/10.1016/S0032-5910(03)00118-9.

[45] A. Behkish, Z. Men, J.R. Inga, B.I. Morsi, Mass transfer characteristics in a large-scale slurry bubble column reactor with organic liquid mixtures, Chemical Engineering Science. 57 (2002) 3307–3324. https://doi.org/10.1016/S0009-2509(02)00201-4.

[46] P.G. Hollis, K.G. Clarke, A systematic quantification and correlation of oxygen transfer coefficients and interfacial area in simulated model hydrocarbon-based bioprocesses in stirred tank reactors, Journal of Chemical Technology and Biotechnology. (2016). https://doi.org/10.1002/jctb.4897.

[47] K.G. Clarke, P.C. Williams, M.S. Smit, S.T.L. Harrison, Enhancement and repression of the volumetric oxygen transfer coefficient through hydrocarbon addition and its influence on oxygen transfer rate in stirred tank bioreactors, Biochemical Engineering Journal. 28 (2006) 237–242. https://doi.org/10.1016/j.bej.2005.11.007.

[48] L.D. Correia, K.G. Clarke, Measurement of the overall volumetric oxygen transfer coefficient in alkane-aqueous dispersions, Journal of Chemical Technology & Biotechnology. 84 (2009) 1793–1797. https://doi.org/10.1002/jctb.2246.

[49] G.K. Gakingo, K.G. Clarke, T.M. Louw, A numerical investigation of the hydrodynamics and mass transfer in a three-phase gas-liquid-liquid stirred tank reactor, Biochemical Engineering Journal. (2020). https://doi.org/10.1016/j.bej.2020.107522.

[50] A. Dejaloud, F. Vahabzadeh, A. Habibi, Hydrodynamics and oxygen transfer characterization in a net draft tube airlift reactor with water-in-diesel microemulsion, Fuel Processing Technology. 171 (2018) 265–276. https://doi.org/10.1016/j.fuproc.2017.11.027.

[51] C.L. Hyndman, F. Larachi, C. Guy, Understanding gas-phase hydrodynamics in bubble columns: a convective model based on kinetic theory, Chemical Engineering Science. 52 (1997) 63–77. https://doi.org/10.1016/S0009-2509(96)00387-9.

[52] A. Prakash, A. Margaitis, H. Li, M.A. Bergougnou, Hydrodynamics and local heat transfer measurements in a bubble column with suspension of yeast, Biochemical Engineering Journal. 9 (2001) 155–163. https://doi.org/10.1016/S1369-703X(01)00137-1.

[53] N.A. Kazakis, A.A. Mouza, S. V. Paras, Coalescence during bubble formation at two neighbouring pores: An experimental study in microscopic scale, Chemical Engineering Science. 63 (2008) 5160–5178. https://doi.org/10.1016/j.ces.2008.07.006.

[54] J. Chalupa, O. Novák, M. Halecký, J. Bárta, E. Kozliak, Thermophilic waste air treatment of n-alkanes in a two-phase bubble column reactor: the effect of silicone oil addition, Journal of Chemical Technology and Biotechnology. 96 (2021) 1682–1690. https://doi.org/10.1002/jctb.6693.

[55] S. Li, S. Huang, J. Fan, Effect of Surfactants on Gas Holdup in Shear-Thinning Fluids, International Journal of Chemical Engineering. 2017 (2017). https://doi.org/10.1155/2017/9062649.

[56] G. Besagni, F. Inzoli, G. De Guido, L.A. Pellegrini, The dual effect of viscosity on bubble column hydrodynamics, Chemical Engineering Science. 158 (2017) 509–538. https://doi.org/10.1016/j.ces.2016.11.003.

[57] J.W.A. De Swart, R.E. van Vliet, R. Krishna, Size, structure and dynamics of “large” bubbles in a two-dimensional slurry bubble column, Chemical Engineering Science. 51 (1996) 4619–4629. https://doi.org/10.1016/0009-2509(96)00265-5.

[58] R. Krishna, J.W.A. De Swart, J. Ellenberger, G.B. Martina, C. Maretto, Gas Holdup in Slurry Bubble Columns: Effect of Column Diameter and Slurry Concentrations, AIChE Journal. 43 (1997) 311–316. https://doi.org/10.1002/aic.690430204.

[59] F. Azgomi, C.O. Gomez, J.A. Finch, Correspondence of gas holdup and bubble size in presence of different frothers, International Journal of Mineral Processing. 83 (2007) 1–11. https://doi.org/10.1016/j.minpro.2007.03.002.

[60] C. Leonard, J.H. Ferrasse, O. Boutin, S. Lefevre, A. Viand, Bubble column reactors for high pressures and high temperatures operation, Institution of Chemical Engineers, 2015. https://doi.org/10.1016/j.cherd.2015.05.013.

[61] R. Maceiras, E. Álvarez, M.A. Cancela, Experimental interfacial area measurements in a bubble column, Chemical Engineering Journal. 163 (2010) 331–336. https://doi.org/10.1016/j.cej.2010.08.011.

[62] H.M.M. Letzel, J.C.C. Schouten, R. Krishna, C.M.M. van den Bleek, Gas holdup and mass transfer in bubble column reactors operated at elevated pressure, Chemical Engineering Science. 54 (1999) 2237–2246. https://doi.org/10.1016/S0009-2509(98)00418-7.

[63] G. Besagni, P. Brazzale, A. Fiocca, F. Inzoli, Estimation of bubble size distributions and shapes in two-phase bubble column using image analysis and optical probes, Flow Measurement and Instrumentation. 52 (2016) 190–207. https://doi.org/10.1016/j.flowmeasinst.2016.10.008.

[64] G. Besagni, F. Inzoli, Influence of internals on counter-current bubble column hydrodynamics: Holdup, flow regime transition and local flow properties, Chemical Engineering Science. 145 (2016) 162–180. https://doi.org/10.1016/j.ces.2016.02.019.

[65] G. Besagni, N.G. Deen, Aspect ratio of bubbles in different liquid media: A novel correlation, Chemical Engineering Science. (2019). https://doi.org/10.1016/j.ces.2019.115383.

[66] J.Y. Kim, B. Kim, N.S. Nho, K.S. Go, W. Kim, J.W. Bae, S.W. Jeong, N. Epstein, D.H. Lee, Gas holdup and hydrodynamic flow regime transition in bubble columns, Journal of Industrial and Engineering Chemistry. 56 (2017) 450–462. https://doi.org/10.1016/j.jiec.2017.07.043.

[67] K.G. Clarke, M.M. Manyuchi, Methodology for advanced measurement accuracy of the overall volumetric oxygen transfer coefficient with application to hydrocarbon-aqueous dispersions, Journal of Chemical Technology and Biotechnology. 87 (2012) 1615–1618. https://doi.org/10.1002/jctb.3853.

[68] T.H. Ngo, A. Schumpe, Oxygen absorption into stirred emulsions of n-alkanes, International Journal of Chemical Engineering. 2012 (2012). https://doi.org/10.1155/2012/265603.

[69] J.L. Rols, G. Goma, Enhancement of oxygen transfer rates in fermentation using oxygen-vectors, Biotechnology Advances. 7 (1989) 1–14. https://doi.org/10.1016/0734-9750(89)90900-2.

[70] A. Mandalenaki, N. Kalogerakis, E. Antoniou, Production of high purity biosurfactants using heavy oil residues as carbon source, Energies. 14 (2021). https://doi.org/10.3390/en14123557.

[71] J. Fernández-Martínez, M.J. Pujalte, J. García-Martínez, M. Mata, E. Garay, F. Rodríguez-Valera, Description of Alcanivorax venustensis sp. nov. and reclassification of Fundibacter jadensis DSM 12178T (Bruns and Berthe-Corti 1999) as Alcanivorax jadensis comb. nov., members of the emended genus Alcanivorax, International Journal of Systematic and Evolutionary Microbiology. 53 (2003) 331–338. https://doi.org/10.1099/ijs.0.01923-0.

[72] J.S. Sabirova, A. Becker, H. Lünsdorf, J.M. Nicaud, K.N. Timmis, P.N. Golyshin, Transcriptional profiling of the marine oil-degrading bacterium Alcanivorax borkumensis during growth on n-alkanes, FEMS Microbiology Letters. 319 (2011) 160–168. https://doi.org/10.1111/j.1574-6968.2011.02279.x.

[73] W.R. Abraham, H. Meyer, M. Yakimov, Novel glycine containing glucolipids from the alkane using bacterium Alcanivorax borkumensis, Biochimica et Biophysica Acta - Lipids and Lipid Metabolism. 1393 (1998) 57–62. https://doi.org/10.1016/S0005-2760(98)00058-7.

[74] M. Barbato, A. Scoma, F. Mapelli, R. De Smet, I.M. Banat, D. Daffonchio, N. Boon, S. Borin, Hydrocarbonoclastic alcanivorax isolates exhibit different physiological and expression responses to N-dodecane, Frontiers in Microbiology. 7 (2016) 1–14. https://doi.org/10.3389/fmicb.2016.02056.

[75] N. Duerr-Auster, R. Gunde, R. Mäder, E.J. Windhab, Binary coalescence of gas bubbles in the presence of a non-ionic surfactant, Journal of Colloid and Interface Science. 333 (2009) 579–584. https://doi.org/10.1016/j.jcis.2009.01.016.

[76] S. Takagi, T. Ogasawara, Y. Matsumoto, The effects of surfactant on the multiscale structure of bubbly flows, Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences. 366 (2008) 2117–2129. https://doi.org/10.1098/rsta.2008.0023.

[77] A. Tzounakos, D.G. Karamanev, A. Margaritis, M.A. Bergougnou, Effect of the surfactant concentration on the rise of gas bubbles in power-law non-Newtonian liquids, Industrial and Engineering Chemistry Research. 43 (2004) 5790–5795. https://doi.org/10.1021/ie049649t.

[78] A.B. Gandhi, J.B. Joshi, V.K. Jayaraman, B.D. Kulkarni, Development of support vector regression (SVR)-based correlation for prediction of overall gas hold-up in bubble column reactors for various gas–liquid systems, Chemical Engineering Science. 62 (2007) 7078–7089. https://doi.org/10.1016/j.ces.2007.07.071.

[79] A.B. Gandhi, P.P. Gupta, J.B. Joshi, V.K. Jayaraman, B.D. Kulkarni, Development of unified correlations for volumetric mass-transfer coefficient and effective interfacial area in bubble column reactors for various gas-liquid systems using support vector regression, Industrial and Engineering Chemistry Research. 48 (2009) 4216–4236. https://doi.org/10.1021/ie8003489.