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Bioprocess Design: The GEOGAS Project

Bioremediation of geothermal gases and SCP production with HOX/SOX bacteria

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BIOPROCESS DESIGN: THE GEOGAS PROJECT

Bioremediation of geothermal gases and SCP production with HOX/SOX bacteria

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A 30 credit units Master´s thesis

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You can know the name of a bird in all the languages of the world, but when you're finished, you'll know absolutely nothing whatever about the bird... So let's look at the bird and see what it's doing — that's what counts. I learned very early the difference between knowing the name of something and knowing something.

Richard Feynman (1918-1988)

ABSTRACT

Bioprocess engineering is a field of science which lately has been experiencing huge growth. Progress in genetic engineering and microbiology, as well as engineering improvements, allowed overcoming the limits, both technical and economical, experienced by industrial processes as recently as ten years ago. Still, bioprocess design and scale-up are highly interdisciplinary fields which rely heavily on previous work in the area. However, for novel processes, there is not much relevant research, which makes the introduction of new bioprocesses challenging. One such case is the GEOGAS project, which aims at utilization of sulfur- (SOX) and hydrogen-oxidizing (HOX) bacteria for simultaneous abatement of hydrogen sulfide and carbon dioxide from geothermal power plants and production of single-cell proteins (SCP). In this work bioprocess design (and engineering) principles are introduced to provide a GEOGAS-oriented framework for tackling new process introduction and scale-up. Further on, in the case study of the Project, the focus is placed on determining crucial factors and issues which could possibly be encountered during scale-up. The obtained results show that the current shape of the design is not yet satisfactory; however, it presents a possibly big gap for tackling numerous pollution and waste disposal problems. Finally, a brief discussion on possible project follow-up and development is presented.

ACKNOWLEDGEMENTS

It has been quite a challenge working on this project and it would not have come to an end without the help and support of numerous people, whom I would like to thank.

First of all, I am very grateful to Jóhann, my Academic Advisor, for the amount of patience and understanding he showed during those last couple of months, but also for the hours spent on valuable discussions and giving a helping hand during the crucial moments. I would also like to thank my Project Advisors – Arnþór and Jakob for the free hand and trust I was given in the work.

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Finally, this work could not have been completed without the support of my fellow students, colleagues and friends, who I owe a lot. However, some of them need more attention here. Patience, acceptance and understanding, invaluable advice, late night talks, time spent together, opportunity to wind down, but also the needed push you gave me during my stay here – Paweł, Krista, Jasiu, Erin, Magda, Mati, Gabor – for all that and more, huge thank you is still not enough.

In addition to that I cannot forget about all those who still put so much faith in me. There are far too many to count, but I know that if they read this, they will know…

Lastly, I would like to express my deepest gratitude to my family, especially Mum, for the unrelenting attitude and unconditional support, which led me to the point where I am now.

PREFACE

Hardly ever do we realize that bioprocesses were always present in our lives. The topic, until very recently, stood somewhere on the sidelines. What brought it to the spotlight was a rapid growth in the genetic engineering and, more recently, biofuels industry, however mainly in the context of discussions about ethics and environment abuse. Hence, it is not so surprising that there are still very few well educated people in the topic. The field spans over an extremely wide scope of different subjects, which does not make it easy for experts with a narrower field of expertise to communicate with others of a different proficiency, not to mention to cover it all by one person alone. For that reason I was very reluctant to undertake this kind of topic.

Behind the whole work lies the GEOGAS project analyzed in the text. The main objective was to facilitate the scale-up of the project throughout this study, trying to address some of the issues which are typical for this kind of activity. The other aim was to use the project as a base to provide some kind of a framework for tackling new "bio-design" problems, accessible for people from both technical and biological backgrounds. Only after trying to cover the whole range of the subject did I realize that it is virtually impossible. Because of that, the "introductory" part, even if it does not seem so, had to be substantially reduced. Therefore, what is left are only the most basic topics; moreover, only those relevant to the scope of the project – namely, airlift bioreactors and microbial sulfur oxidation.

The study has been divided into two parts. The first introduces the basic concepts and provides some good practice examples from the field of bioprocess engineering and design. The last two last chapters aim at the use of the previously introduced background to critically analyze the GEOGAS project.

Finally, even though there are certain flaws in the taken approach, it is hoped that the text will be of use for both practitioners, as well as novices, in such a promising field as novel bioprocess design.

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NOTATION

All the abbreviations and symbols used, if not stated in the text after their first use, can be found below.

If not stated differently, the term - "the project" refers to the GEOGAS project.

Bioreactor, bioreaction and bioprocess engineering are not the same fields of science, yet for simplification, all will be referred to in common terms as bioprocess engineering, which has the biggest scope of them all.

Fermenter, for the scope of this work, will be regarded as a fermentation bioreactor or simply (bio)reactor.

Air-lift and bubble-column reactors differ slightly in the principle of operation discussed in the chapter on airlift reactors, yet the terms will not be strictly distinguished.

Abbreviation	
ALR	Airlift reactor
ATP	Adenosine triphosphate
CCS	Carbon capture and storage
CFD	Computational fluid dynamics
CSTR	Continuously stirred tank reactor
DOE	Department of Energy
FAO	Food and Agriculture Organization
GH	Gas holdup
HOX	Hydrogen-oxidizing
MIT	Massachusetts Institute of Technology
NCG	Non-condensable gases
NG	Natural gas
NREL	National Renewable Energy Laboratory
O&M	Operation and maintenance
OTR	Oxygen transfer rate
RNA	Ribonucleic acid
SCP	Single-cell protein
SOB	Sulfur-oxidizing bacteria
SOX	Sulfur-oxidizing

Superscript	
·	Flux
‘	Differential
–	Mean value, Average
*	Saturation conditions; whole mixture

Subscript	
1, 2	Ordering numbers
A	Component
aer	Aerobic
anaer	Anaerobic
BM	Biomass
CO2	Refers to CO_2
G	Gas
H2	Refers to H_2
H2S	Refers to H_2S
I, i	Interface; component's index; inhibition
L	Liquid
M	Molar
m	Maximal
n	Exponent in power law
O2	Refers to O_2
P	Product
Q	Energy; heat
S	Solid; substrate
th	Thermal

Symbol	
A	Area
a	Interfacial area
C	Concentration; integration constant
c	concentration
c-mol, C-mol	Carbon-mole substrate
D	Diffusivity
d	Diameter
E+X	Scientific notation, $\times 10^{+X}$
F	Force
g	Gravitational constant
G	Gibbs free energy
H	Henry's constant; enthalpy; height
j, J	Molar flux; velocity
k	Mass/heat transfer coefficient
k_La	Overall mass transfer coefficient
L	Length (dimension),

Symbol	
m	Mass; constant in power law
M	Molar mass
p	Pressure
ppmv	Parts per million, volume
Q	Energy; heat
r	Uptake/reaction rate
R	Individual gas constant
s	Substrate concentration
T	Time (dimension); temperature, absolute
t	temperature
u	velocity
V	Volume; velocity
v/v	Volumetric ratio
vol.	Volume
wt.	Weight; weight basis
X	Biomass concentration
x, y, z	Coordinates; variables
$Y_{A/B}$	Yield/uptake coefficient of component A in respect to component B
δ	Differential length
η	Efficiency
μ	Specific growth rate
ρ	Density
σ	Surface tension
τ	Shear stress
χ	Association parameter (in equation for diffusion coefficient)

1 NOVEL BIOPROCESSES

The end of the twentieth century experienced huge progress in science especially in biotechnology. New opportunities opened and most of them still lie unused; The underlying cause of which being associated with the diffusion of know-how and money.

New bioprocess development used to be tedious and long. The timeline – from initial idea to product market introduction – expanded to as much as a decade, as illustrated in Figure 1.1 adapted from (Nielsen, Villadsen and Liden, 2003).

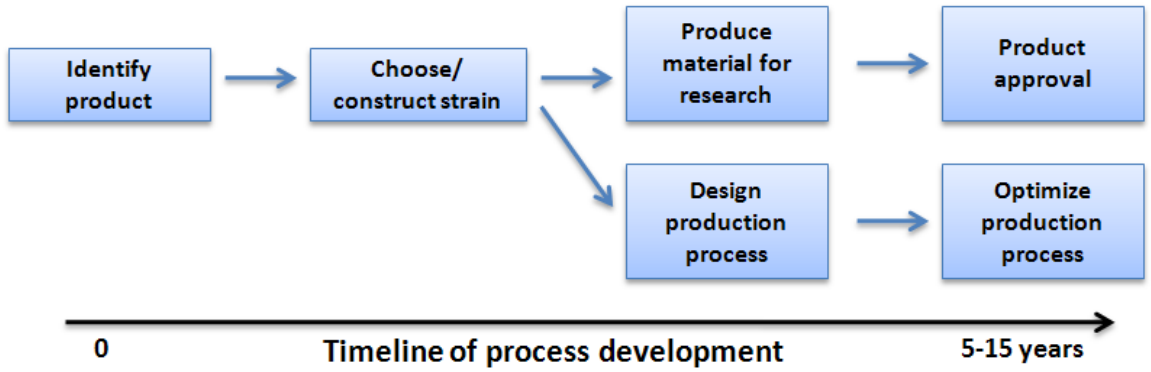


Figure 1.1 Timeline of development of a bio-product from fermentation

Currently, however, the timeline can be as short as 3-4 years (Wesselingh, Kiil and Vigild, 2007). However there are still several steps which apply to any new product development:

- Analysis of current market;
- Finding demand or a niche for the product;
- Concept selection and product specification;
- Process design;
- Cost estimation and major cost-determining factors identification;
- Small-scale analysis;
- Scale-up and process optimization;
- Market introduction;
- Process and future product development.

In this framework the GEOGAS project, introduced more thoroughly further, will be analyzed.

1.1 Project background

Geothermal energy is abundant and of high quality in Iceland, thus it has a big share in the energy portfolio of the country. It is said to be one of the cheapest sources of renewable energy in the long run, but it is still not entirely environmentally neutral. Even though they are usually not so significant, there are some emissions and environmental impacts connected with geothermal power plant operation. The most common are CO_2 emissions from the boreholes, which are not yet accounted as industrial emissions in the scope of the Kyoto protocol. However, a bigger concern is hydrogen sulfide (H_2S) presence in the rejected geothermal gas. Apart from being lethal at relatively low concentrations and causing corrosion and sulfur deposition issues, its odor can be very disturbing for all the people in the vicinity of the plant.

The most common methods of H_2S (and other sour gases as well) removal involve a mixture of physical and chemical processes – typically washing or solving with some kind of reaction with basic compounds. However the biggest advantage of geothermal power – its exceptionally low O&M costs – could be greatly reduced by the need for deployment of such methods.

Taking into account the amount of geothermal power in Iceland and the problematic emissions of NCG (Non-Condensable Gases) connected with it, especially hydrogen sulfide, different kinds of non-chemical clean-up technologies were (and are) being investigated. On the other hand, there can also be a significant amount of hydrogen and carbon dioxide present in the gas stream rejected from the geothermal power plants. Those in turn are a perfect energy and carbon source for bacteria. Making use of those could provide simultaneous remediation of geothermal gases – otherwise vented into the atmosphere – and production of microbial biomass, which is currently referred to as SCP (single-cell protein). For that purpose, the GEOGAS project was established.

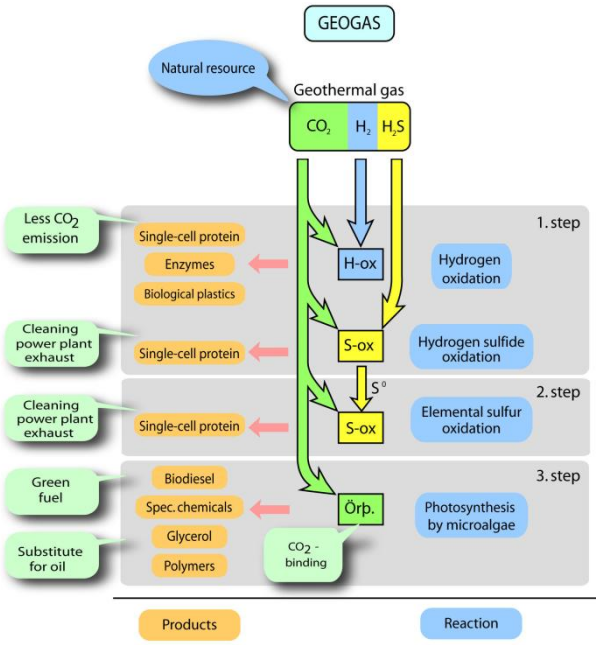


Figure 1.2 The GEOGAS project outline (Copyright, Prokatin ehf., 2008)

The concept depicted in Figure 1.2 shows the consecutive research and development parts of the GEOGAS project, which focuses on the biological use of geothermal gas. The final aim of the project is to develop technology for a stepwise utilization of the components of the geothermal gas for the growth of microorganisms such as bacteria and microalgae. The microbial biomass produced in such a way becomes the source of valuable products such as single-cell protein (SCP), bio-derived fuels and specialty chemicals (Ævarsson, 2008).

Still, there are many technological hurdles to overcome. There are very few examples of successful SCP production and gaseous fermentations on an industrial scale. What is more, hydrogen sulfide is an uneasy compound to deal with and sulfur and/or sulfuric acid, which are the by-products of the process, have to be properly separated from the main product and somehow utilized or disposed of – adding up to the complexity of the process.

The following chapters are intended to shed more light on possible issues in process development, with focus on issues relevant to the project.

1.2 Single-cell protein

Even thought there are discrepancies as to when exactly the term was first coined (Anupama, 2000), (Litchfield, 1978), it was around the end of the sixties at MIT when nonviable microorganism cells grown for consumption, because of their valuable protein content, started to be referred to as single-cell protein (SCP) instead of "microbial protein". Even though over forty years have passed since then, there are very few examples of SCP being used as food – for human consumption – rather than feed. Numerous concepts for the development of the technology and the provision of a cheap protein source, so badly needed, still have not yet reached the stage of full commercial availability. Thus, this chapter will mainly focus on the general characteristics and uses of SCP, examples of industrial scale processes for single-cell protein production and the hurdles that have to be overcome to allow for free and full scale market introduction.

1.2.1 Human SCP consumption

Because of the rapidly growing population and ever-increasing resource consumption, scarcity of food gains more importance as a global problem (Gilbert, 2002). The trial of improving the situations of millions of impoverished people calls for a search for cheaper, alternative protein sources. Microbes, due to their rapid, in comparison to other, higher organisms, growth rate are now thought to become the possible solution for the problem. Though having microbes as a food source may be very unappealing for most, humans have already utilized this source for millennia. A very good example can be found in the seafood-rich Japanese and Pacific region cuisine, in the form of algae. Furthermore, alcoholic beverages, cheese, yogurt, soya sauce, bread and more have been, intentionally or not, consumed along with the biomass which was used for its production (Tuse, 1984), (Anupama, 2000). Some cultures even used to harvest the microorganisms for consumption directly, like Aztecs did with the algae *Spirulina* (Anupama, 2000), (Singh, 1998). Yet, the current population is still very reluctant to agree on the consumption of SCP. The main reasons for that are:

- Distrust in safety of single-cell protein consumption by humans;
- Lack of general public acceptance and bias against bioengineered products;

- Appropriateness of nutritional value and the amino acid composition for human consumption;
- The final product is not as appealing and its characteristics are less desirable compared to common staple foods.

1.2.2 SCP production - substrates

All different types of substrates and microorganism were shown to be suitable for the production of SCP (Litchfield, 1978), yet for current industrial production the main focus is on inexpensive substrates and bacteria and fungi as the biological producer, for their high growth rate and protein content. The most common choices for substrates will be investigated subsequently.

Gaseous hydrocarbons

Natural gas used to be of interest as a substrate for fermentation in SCP production for its favorable characteristics as a carbon and energy source. The main bacteria strains that were reported in literature to grow on NG, and that were suitable for single-cell protein production, belong to genera like *Methylococcus*, *Methanomonas* and *Pseudosomonas* (Litchfield, 1978). A continuous operation mode was preferred as higher productivities (wt. biomass/L^3T) are obtainable and recirculation of non-utilized substrate is possible. High productivities and yield coefficients could be achieved if the problem of limiting oxygen and methane mass transfer to the bacterial cells could be handled. Other typical issues met by plants operating on methane are the requirement for explosion hazard prevention and high heat generation during bacterial growth, both of which sharply raise capital investment costs. Another economical problem lies in the NG itself, as there are few places left where it can be found for cheap with the means to use it on-site or transport it. One example of a successful process was the Bioprotein process developed by Norferm and, more recently, UniProtein® made by UniBio A/S and described in (UniBio A/S, n.d.) and (Villadsen, n.d.).

Liquid hydrocarbons

Out of all the different types of hydrocarbons, n-alkanes utilized aerobically seem to have the biggest potential for industrial scale application. Crude oil, fuel oil, kerosene and other liquid oil derivates were studied, but their results were not as promising (Litchfield, 1978). Contrarily to gaseous hydrocarbons, liquid hydrocarbons used as substrates were quite often utilized in batch mode, especially when operated on yeast culture (Litchfield, 1978). As in the previous case, general issues which need to be addressed involve: oxygen transfer, mass transfer of the substrate to the cell and heat generation. Apart from that, the hydrocarbons are poorly miscible with water and the obtained product has to be purified (Israelidis, n.d.).

Methanol

Methanol was a substrate of special interest for SCP production in the 1970s and 1980s. The main advantage over other potential carbon sources is its high miscibility with water, which removes the need for protein purification (Rai University, n.d.). However, there are also some issues connected with the use of methanol as a substrate. Most of all, microbial tolerance for methanol is rather low (in the range of a percent) and its oxygen demand and heat generation are high. The Pruteen process running on this substrate deployed one of the

biggest airlift fermenters, yet it was shut down due to a rise in the price of methanol, which accounted for over the half of the running cost of the plant (Rai University, n.d.).

Other substrates

There were many other trials that used organic substrates (like whey in the Bel process), industrial waste streams (spent sulfite liquor – Pekilo process) or different microorganisms (mainly fungi) for SCP production. More information regarding those can be found in the literature: (Lee, 2008), (Rai University, n.d.), (Litchfield, 1978).

Nutritive value

As in the case of any source of food or feed, its value is based on its composition. SCP is especially rich in proteins, but there are also other components present, such as:

- Carbohydrates;
- Fats;
- Amino acid profile;
- Nutrients and vitamins;
- Cell wall components, nucleic acids, nitrogen.

All of the above should be carefully analyzed before using SCP as a food/feed source or supplement. Special attention has been given by FAO to the referenced amino acid profile. Examples of SCP complying with those can be found in (Single Cell protein, n.d.).

Rules of thumb state that bacterial SCPs (in comparison with algae and yeast) have the highest protein content by dry weight, but also the highest nucleic acid content. A table taken from (Anupama, 2000) shows the typical composition of different kinds of SCP in accordance with the abovementioned characteristics:

Table 1.1 SCP composition by microorganism type

Component	Percentage composition of weight		
	Algae	Fungi	Bacteria
True proteins	40–60	30–70	50–83
Total nitrogen (Protein + nucleic acids)	45–65	35–50	60–80
Lysine	4.6–7.0	6.5–7.8	4.3–5.8
Methionine	1.4–2.6	1.5–1.8	2.2–3.0
Fats/Lipids	5–10	5–13	8–10
Carbohydrate	9	NA	NA
Bile pigment and Chlorophyll	6	NA	NA
Nucleic acids	4–6	9.70	15–16
Mineral salts	7	6.6	8.6
Amino acids	NA	54	65
Ash	3	NA	NA
Moisture	6.0	4.5–6.0	2.8
Fiber	3	NA	NA

1.2.3 Issues to overcome in bioprotein production

If the protein is to either be fed to animals or used as food for people, it has to be safe. For bacterial SCP this means a reduction of RNA content from 10-15% to, at most, 2% (wt.) (Rai University, n.d.). However, in most cases this is an insufficient amount of processing. Possible product contamination, which includes toxins (bacterial and fungal), pathogens and sometimes even the substrates (i.e. hydrocarbons) has to be controlled and avoided (Litchfield, 1978). Moreover, there are technical issues connected with production – mainly high oxygen demand and heat generation, substrate handling issues and some others (Rai University, n.d.). All of them put a lot of strain on proper reactor design.

The economics also play a major role, as most of the processes ceased operation due to economic problems (Lee, 2008). Several options for reduction of cost-related issues were proposed (Rai University, n.d.):

- Cheaper process in the upstream part, i.e. inexpensive substrates;
- Genetic modification of microorganisms for higher process efficiency;
- Use of the product for human consumption rather than just for feed;
- Multi-product processes, preferably with some high-value products;
- Lowering downstream processing costs – reduction of RNA levels, removal of necessity for final product purification.

2 BIOPROCESS ENGINEERING PRINCIPLES

The terms bioprocess design and bioprocess engineering are both a result of insufficiency of, and progress in the field of chemical engineering. Currently all the industrial and microbial processes requiring constantly improving yields, productivity and cost reduction cannot be handled anymore by chemical engineers alone. Also for biochemical engineers, who are usually employed to tackle the introduction of novel processes, the area of biological process engineering is not a main focus (Nielsen, Villadsen and Liden, 2003). Thus, the need for joint venture of the two abovementioned groups resulted in the creation of a new field on the border of microbiology, chemistry and engineering – namely – bioprocess engineering.

Because of the expansive scope of the subject, this chapter will focus only on the introduction of major concepts necessary in an analysis of the project.

2.1 Bioprocess design – introduction to economics

Economics always provides the final test for any process and, as in the saying – it is better to prevent than cure – careful planning and anticipation of possible issues from the very beginning is necessary.

For bio-production, the revenue comes from product sale. Thus, it is very important to first analyze the market and choose a niche in which there will still be potential demand for the product. Market size, however, depends also on the sale price. An exemplary hierarchy of the prices of different bio-products, adapted from (Doran, 1995), is shown in Figure 2.1.

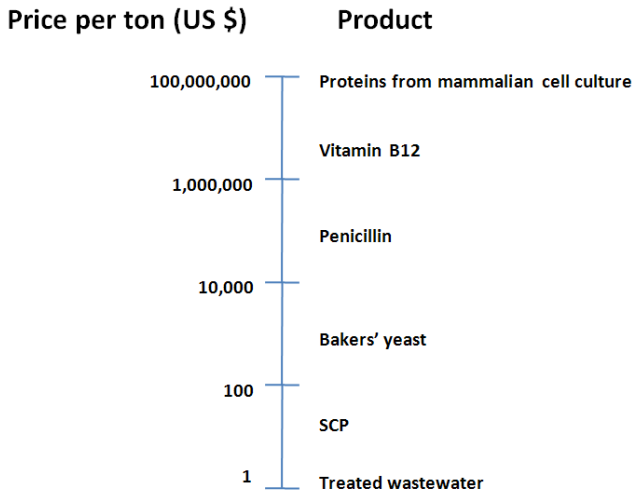


Figure 2.1 Selection of products from bio-processes and their price per ton

On the other hand, there are several factors determining the production cost which diminish the final profit. These can be divided into four major groups according to the part of process development and operation, which has the biggest impact of unit production cost, as shown in Figure 2.2, adapted from (Doran, 1995).

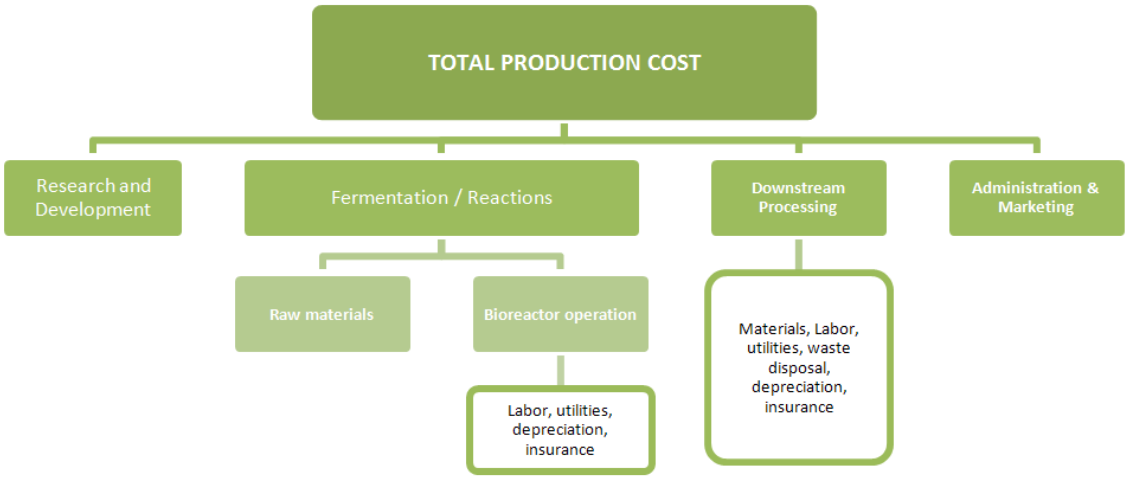


Figure 2.2 Contribution of different production aspects to the final product cost

There are several strategies to reduce the costs, some of which are put together in Figure 2.3 taken from (Doran, 1995). However there are many other possible means. Currently, especially because of the boom for biofuels, there are many efforts being made to use cheap substrates for fermentations. On the other hand, there is much work put into the genetic engineering of strains to obtain recombinant organisms with higher yields and substrate utilization (Yang, et al., 2007). The latter comes into play because separation technologies like distillation (for liquid-liquid separations) or spray drying (reducing water content) are very energy-intensive and can easily overrun the advantage gained by the use of cheap substrates.

Presently outsourcing, especially when scale-up and genetic manipulation is made, is a common practice. Chemical conversion methods are still based mainly on well-proven technologies introduced decades ago. This provides a competitive edge for biological processes, which evolve and develop at an astounding rate.

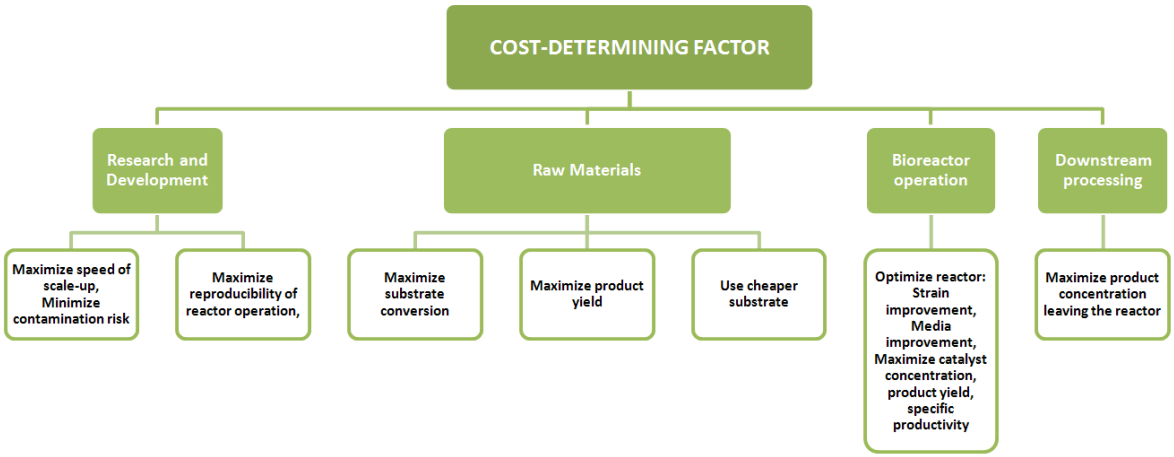


Figure 2.3 Typical solutions for reduction of production costs

2.2 Bioprocess design – general guidelines

A bioprocess can be treated as a complex system combining different biological, chemical, physical and mechanical operations into one entity. This implies a very dense network of not always obvious relations between different components. To make it work in a predictable manner, major interactions between design parameters should be identified. Hence, a certain order on the undertaken steps is forced, as is shown in the diagram below, adapted from (Asenjo and Merchuk, 1995).

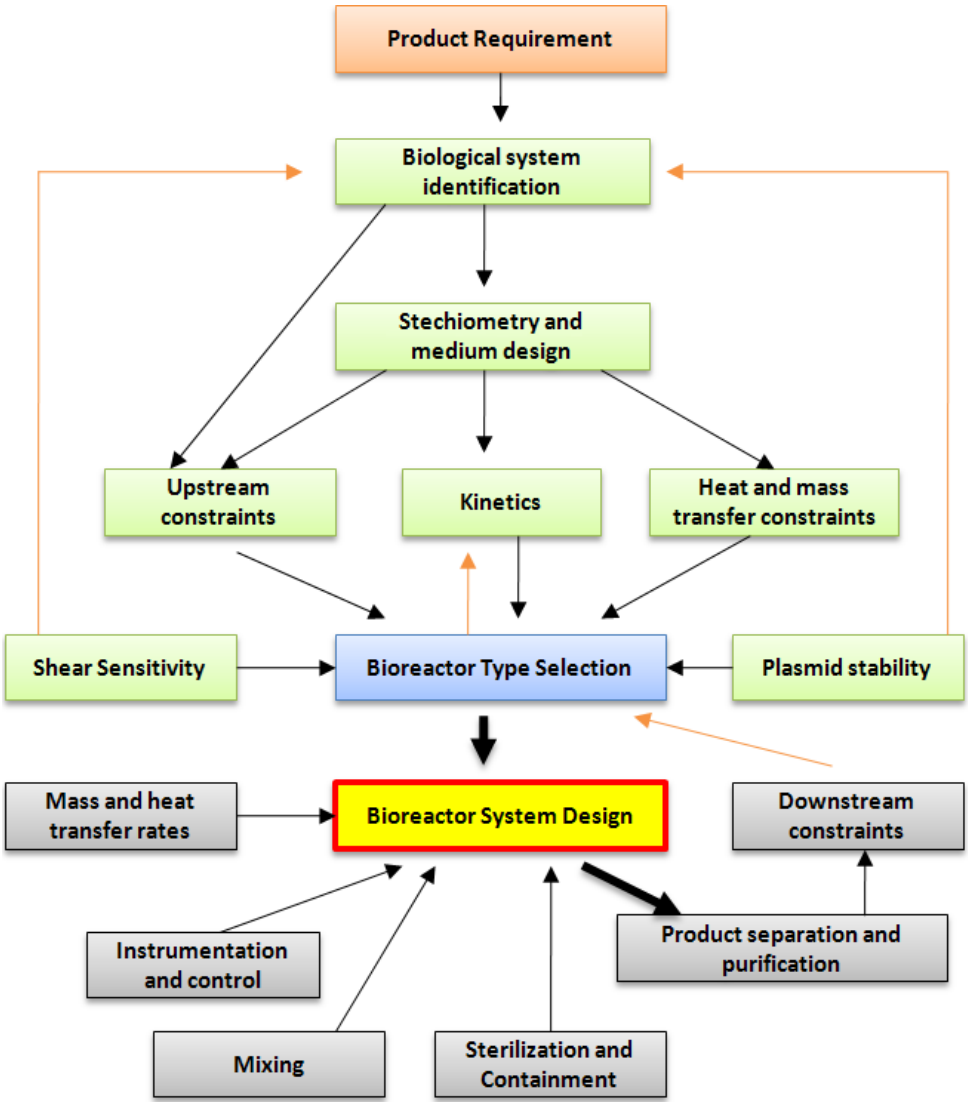


Figure 2.4 Components' interaction network in bioprocess design

2.3 Bioprocess scale-up

Scale-up is a set of activities aimed at the successful increase of the scale of operation of a process, usually by orders of magnitude. In the engineering communities it is often regarded as more of an art than pure science (Nielsen, Villadsen and Liden, 2003). Many different, often unpredictable, phenomena have to be taken into account, while many inherently conflicting but desirable characteristics need to be carefully weighted for a

satisfactory final result. Still, there are some fundamental problems always encountered during scale-up. A brief revision of those issues shall be discussed throughout this chapter.

One can distinguish three fundamental phases of a bioprocess project – from the concept stage to product market introduction. These are:

- Lab scale;
- Pilot scale/plant;
- Industrial scale/commercial plant.

The biggest transition is made between the first and second scale, for many different reasons which will be discussed further, but the most important groups of issues to be integrated into the design of a pilot plant can be represented by a diagram adapted from (Nielsen, Villadsen and Liden, 2003).

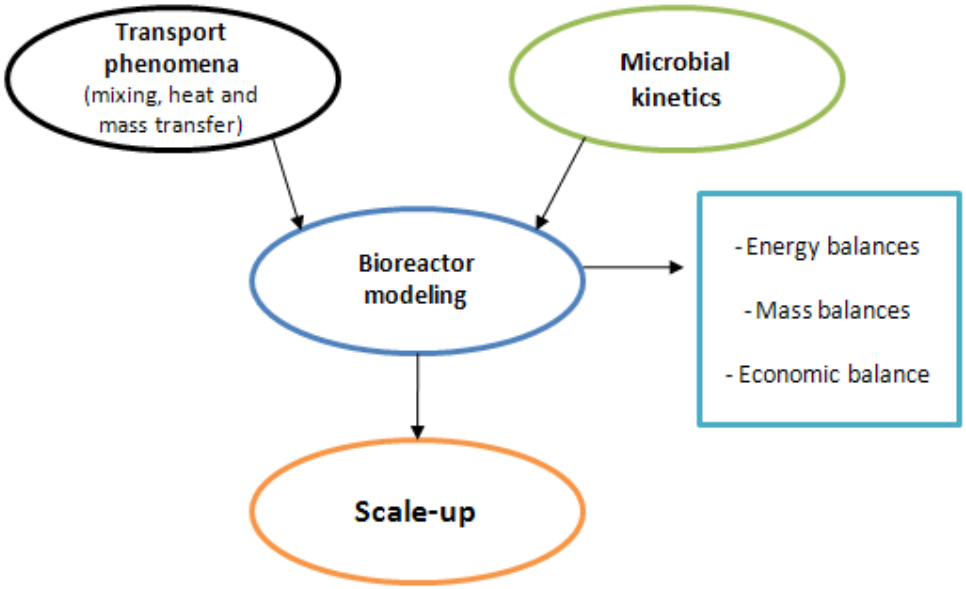


Figure 2.5 Basic approach to scale-up

Bioreactor modeling

Nowadays bioreactor modeling can be greatly facilitated using CFD software or complex mathematical modeling. However, in most cases, there are many parameters to include in the model for sufficient accuracy, which are hardly ever obtained even at the pilot plant scale stage. For that reason, bioreactor modeling and the mathematical approach behind it goes far beyond the scope of this work. More details about the subject are included in work by (Jakobsen, 2008).

For a further, simplified analysis Table 2.1, adapted from (Jakobsen, 2008), which is useful for energy and mass balancing, is presented.

Table 2.1 Typical values of energy and mass yield coefficients

Type of yield coefficient	Dimension	Typical value
$Y_{X/S,aer}$	c-mol / c-mol	0.4-0.7
$Y_{X/S,anaer}$	c-mol / c-mol	0.1-0.2
$Y_{X/O2}$ (glucose)	c-mol / c-mol	1-2
$Y_{X/ATP}$	c-mol / c-mol	0.35
$Y_{Q/O2}$	kJ / mol	380-490
$Y_{Q/CO2}$	kJ / mol	460
$Y_{Q/X,aer}$ (glucose)	kJ / c-mol	325-500
$Y_{Q/X,anaer}$	kJ / c-mol	120-190

Scale-up methodology

In a way the methodology for scale-up does not differ much from the general approach to bioprocess design. The main difference lies, however, in the main focus of the process – not the whole system, but the reactor. If the biological system has already been identified during the lab scale experiments, it can be assumed that, provided the conditions are the same, its behavior is already known. To fulfill that requirement the pilot and industrial scale reactors have to reproduce the same environment as in the small scale lab. The key problem thus comes down to designing the reactor in such a way that it will provide similar conditions to those under which the cell factory operation was investigated. A box diagram shown in Figure 2.6, adapted from (Si-Jing Wanga, 2007), presents a good practice iterative approach to scale-up.

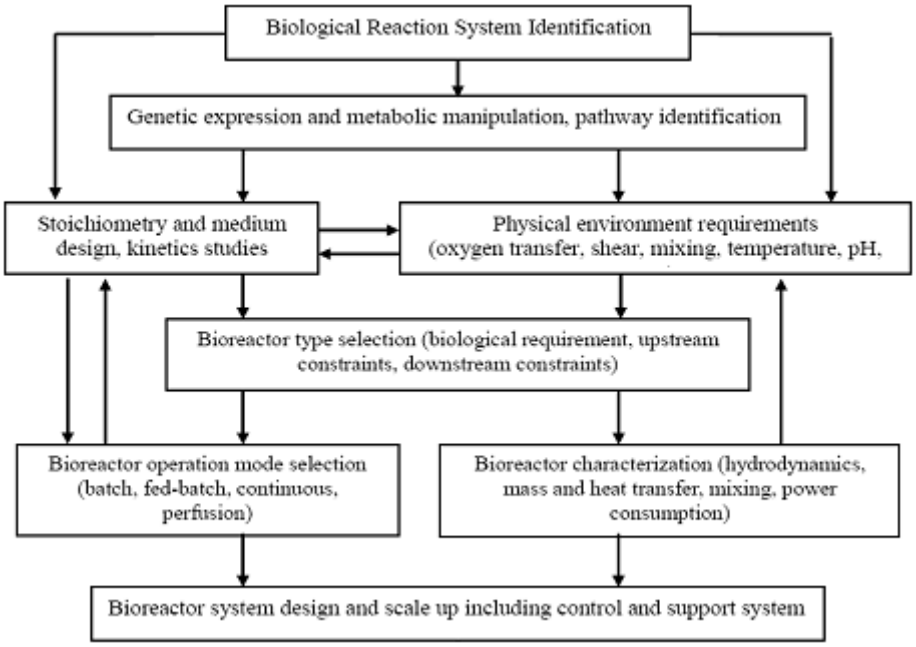


Figure 2.6 An example of scale-up approach

Factors to consider in scale-up

This section focuses on the breakdown of different aspects and processes important for process design and successful scale-up. Please note that it is still just a highlight of the problems associated with data gathering rather than a complete to-do list.

Cell factory

To ensure successful scale-up, the biological system has to be intensively studied during lab scale operation and further on. The basic parameters which need to be determined are cells' growth characteristics and metabolism. However, under those terms there are many aspects hidden. A short listing of factors which should be checked is as follows:

- Biosystem identification (metabolic pathways, genetic studies);
- Optimum growth conditions (pH, temperature, salinity etc.);
- Meta- and catabolic activity (as a function of process parameters);
- Specific growth rate, doubling time;
- Product/substrate yields and uptake rates (maintenance requirements, substrate(s) consumption, product(s) synthesis, by-product(s) formation for calculations of mass and energy balances);
- Shear stress resistance;
- Stress-causing factors (substrate/product inhibition and toxicity);
- Culture stability (over period of time, contamination risk).

Reactor choice

The decision regarding the reactor choice is one of the most important in the whole process design. Hence the following factors should not be neglected by decision-makers:

- Mode of operation (i.e. continuous vs. batch, suspended vs. immobilized system);
- Reactor type (CSTR, airlift, biofilter);
- Mass transfer characteristics (oxygen transfer rate, product removal capacity etc.);
- Mixing characteristics (power input, mixing time);
- Shear (distribution, average/maximum values);
- Operation reliability (possible operation issues, foaming, maintenance ease);
- Operation stability (response to transients, control and monitoring possibility);
- Scalability;
- Cost (initial and of operation).

Control and measurement

- Control of pH, dissolved oxygen, temperature, mixing, supplementation of nutrients.

Media (substrates) requirements and choice

- Provision of substrates at optimal concentrations (based on stoichiometry, metabolism and mass transfer);
- Compliance with upstream constraints (i.e. sterilization requirements, avoiding inhibiting concentrations).

Downstream processing

- By-product disposal;
- Rhelogy of the fluids.

2.4 Microbiology

2.4.1 Sulfur bacteria in bioprocesses

Bacteria are involved in all the biogeochemical cycles. For sulfur, they are involved in all the steps, as presented in Figure 2.7. The sulfur compounds can be either reduced or oxidized in the cycle. The most common reduction step is encountered in wastewater treatment. As a result, H_2S is created, causing considerable problems for the facilities. The project aims at the oxidation of reduced sulfur compounds (i.e. hydrogen sulfide) by bacteria, which can use it as an energy source, thus changing its form into one less harmful or easier to handle.

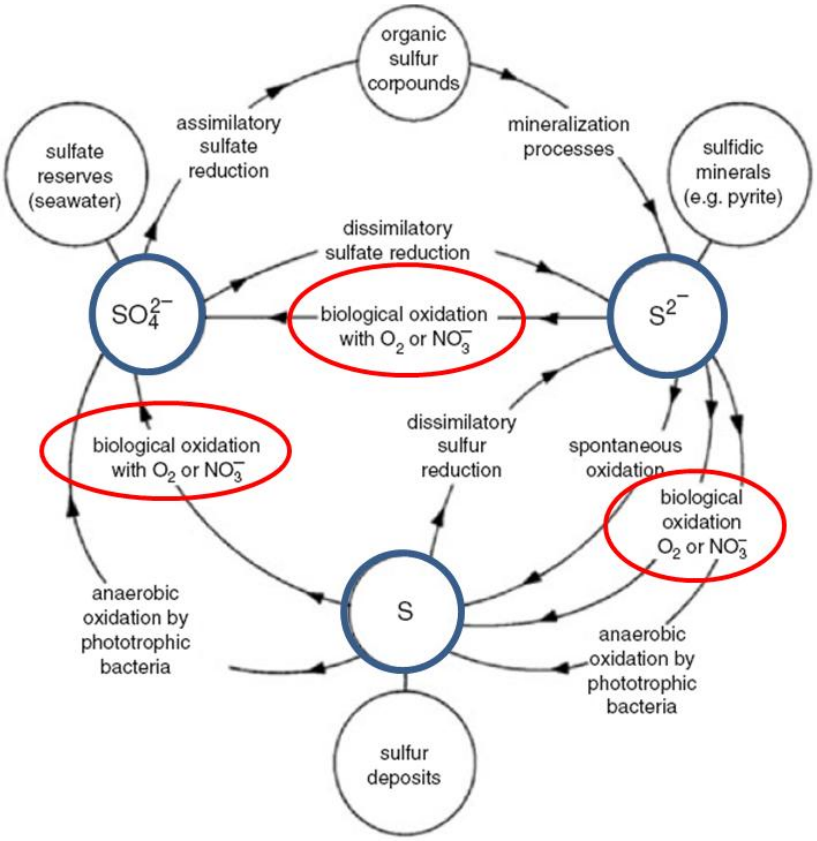


Figure 2.7 Sulfur cycle as in (Robertson and Kuenen, 2006)

Sulfate reducing and sulfide oxidizing bacteria have great potential for industrial and environmental use. Still, just until recently, there was not much interest in harnessing the bacterial ability to use sulfur compounds for growth.

While biogenic production of H_2S by sulfate reducing bacteria creates severe processing and environmental problems for the petroleum industry and agriculture sector, when used in a well-designed process the bacteria could play a pivotal role in the bioremediation of acid mine drainage (Tang, Baskaran and Nemati, 2008). The biological oxidation of reduced and intermediary sulfur compounds can be well applied in coal desulfurization and bioleaching of refractory minerals. Moreover, sulfide oxidizing bacteria are known for their ability to remove H_2S from the oil reservoirs and can be used in biological treatment of sour gases and sulfide laden waters (Lee and Sublette, 1993). Having great potential for environmental and industrial applications, the bacteria of the sulfur cycle have been the subject of numerous studies and extensive overviews, which can be found in the literature: (Cline, et al. 2003), (Tang, Baskaran and Nemati, 2008).

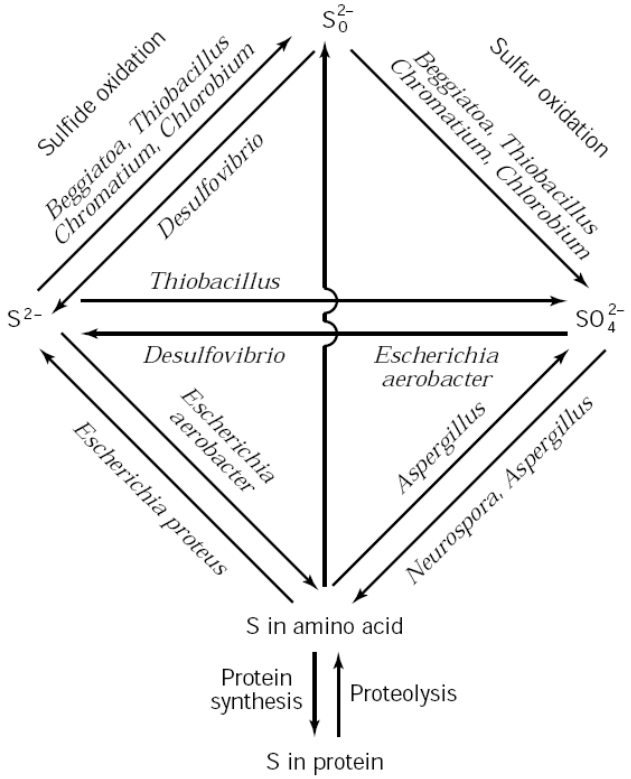


Figure 2.8 Sulfur cycle and the microorganisms involved (Perego and Fabiano, 1999)

Chemolitotrophy

Sulfur bacteria are a wide group of organisms characterized by the ability to use sulfur compounds for growth, which makes most of them chemolitotrophs. On the other hand some of them can use inorganic carbon sources, which proves their autotrophic ability. Therefore sulfur oxidizers can be categorized according to their metabolic mode. Figure 2.9, taken from (Robertson and Kuenen, 2006), shows categorization of all four groups of colorless sulfur bacteria, with bars showing most likely ratio of inorganic to organic substrates favoring each of them.

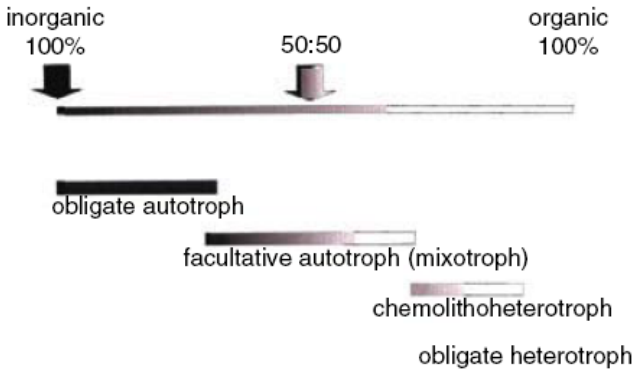


Figure 2.9 Categorization of sulfur oxidizers according to their metabolic mode

2.4.2 Colorless sulfur bacteria

The bacteria belonging to the families of the *Thiobacteriaceae*, *Beggiatoaceae* and *Achromatiaceae* are commonly called the colorless sulfur bacteria. High temperature and/or low pH environments, such as hot acid sulfur springs, sulfide ores, sulfur deposits and some acid soils allow their development as a major population (Robertson and Kuenen, 2006).

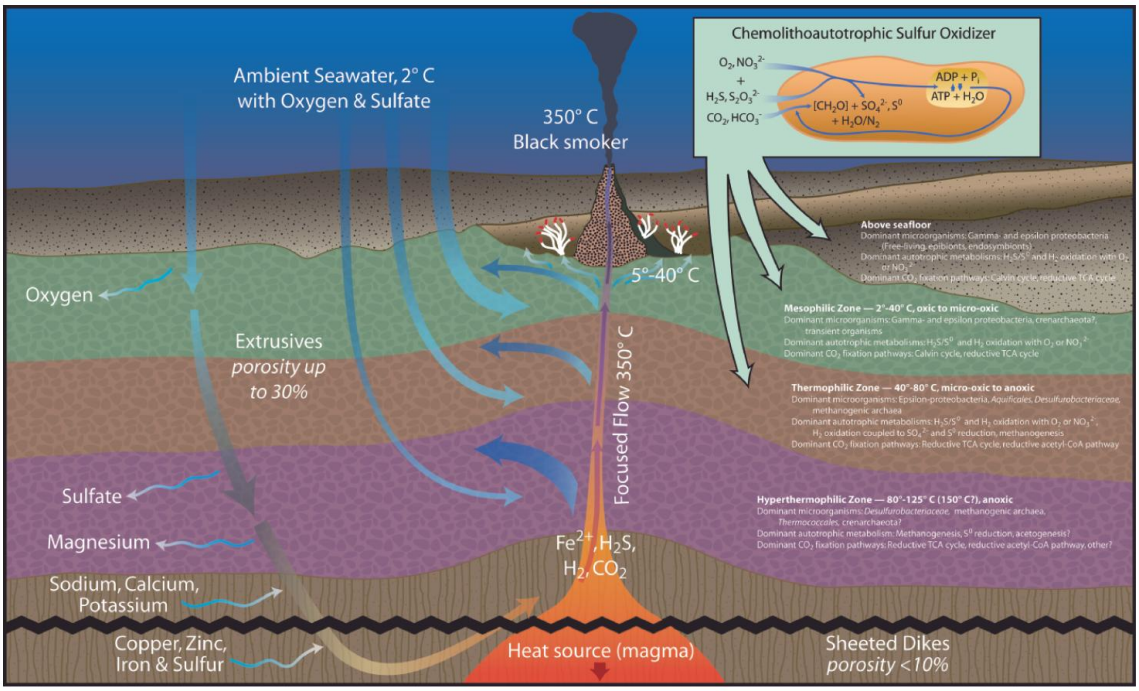


Figure 2.10 Sulfur oxidizers in aquatic habitat (Sievert, et al. 2008)

Bacteria belonging to the group can oxidize a variety of inorganic compounds, like hydrogen and hydrogen sulfide, but also use nitrogen and iron compounds during oxidation (Lengeler, Drews and Schlegel, 1999). Most of the colorless sulfur bacteria can synthesize all cell material from CO_2 and use oxygen as the electron acceptor. Details can be found in the literature: (Lengeler, Drews and Schlegel, 1999), (Robertson and Kuenen, The Colorless Sulfur Bacteria, 2006), (Robertson and Kuenen, The Genus Thiobacillus, 2006).

2.4.3 Kinetics and bacterial growth

Microbial kinetics are probably the most important parameters of the cell factory that are included in the design. It is true that the microbial behavior depends on numerous parameters, mainly pH, temperature and substrate/product concentrations. Nevertheless, if done sensibly, a quantitative description of only the projected reactor conditions is usually enough. For that reason basic concepts and their applicability will be discussed in this section.

Reaction rate and order of kinetics

One can describe the rate of an irreversible reaction in the form of the equation:

$$r = kC^{\alpha}C^{\beta},$$

where k denotes the rate constant and C, concentrations of certain components. Now a division can be made between different forms of the kinetic equations based on the exponents α and β. N-th order kinetics (in respect to a component) have N as an exponent of the concentration in the kinetic equation.

Bacterial kinetics

Bacterial growth can be represented in a similar manner to the kinetic equation:

$$r = \mu X$$

The difference now is that r stands for (substrate) uptake rate, μ for specific growth rate and X for biomass concentration in the reactor. In general, the specific growth rate is not constant, but is dependent on the substrate concentration. This relationship is usually described using the Monod equation in the form:

$$\mu = \mu_m \frac{s}{K_s + s}.$$

In the equation given above, s is substrate concentration, K_s, a (saturation) constant and μ_m stands for the maximum value of the growth rate. The formula is especially useful for the description of batch cultures experiencing a limiting substrate concentration. Thus, the Monod reaction is an example of first order kinetics, as for low concentrations the specific growth rate can be approximated by a linear function of s. Figure 2.11 shows a graphic representation of the situation.

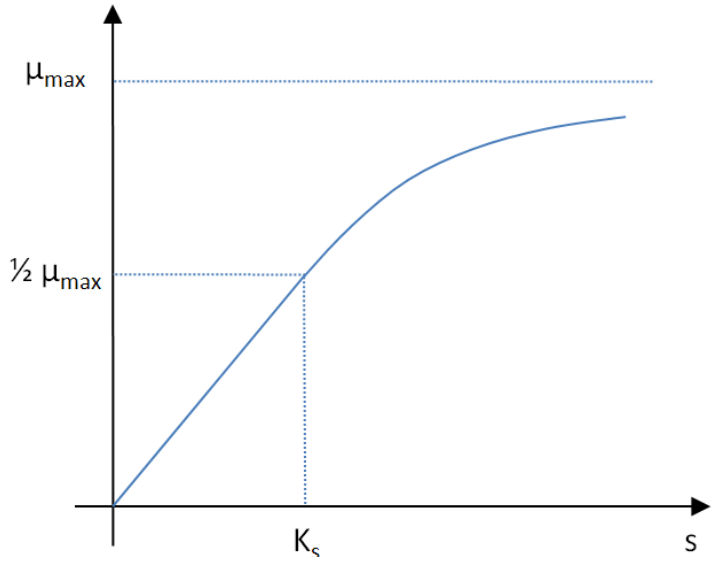


Figure 2.11 Monod reaction for growth-limiting substrate concentration

This basic relation is often used because of its simplicity. However there are many more cases in which it would be better to use more sophisticated models. Table 2.2, which is based on (Dunn, et al. 2003), notes project-relevant possibilities for the kinetics' description. However, due to lack of data and reason stated in Chapter 4.2 they will not be investigated.

Table 2.2 More complex models of bacterial kinetics

Relation	**When applicable**	**Mathematical Formula**
Monod relation for substrate inhibition	If a substrate at sufficiently high concentration becomes limiting	$\mu = \frac{\mu_m S}{(K_s + S + S^2 / K_I)}$
Multiple-Substrate Monod kinetics	When there is more than one substrate, which can be limiting	$\mu = \mu_m \prod_{i=1} \left(\frac{S_i}{K_i + S_i} \right)$
Double Monod kinetics	There are two possible parallel reactions in respect to substrates	$\mu = \mu_m \left(\frac{k_1 S_1}{K_1 + S_1} + \frac{k_2 S_2}{K_2 + S_2} \right) \left(\frac{1}{k_1 + k_2} \right)$
Diauxic Monod	Use of substrate 1 inhibits the use of substrate 2	$\mu = \mu_{m,1} \frac{S_1}{K_1 + S_1} + \mu_{m,2} \frac{S_2}{K_2 + S_2 + S_1^2 / K_I}$

Simplification due to balanced growth

Under ideal conditions, bacteria will be able to maintain their cell composition – and thus operation – constant. Such a situation is referred to as balanced growth (Doran, 1995). It implies that all the substrates are taken up at constant rates. Now, one can use "the black box" approach, in which for an exponential growth phase, the specific growth rate is constant and does not depend on any of the substrates or products. This yields zero-order kinetics in the form of the equation:

$r_s = \mu X$

Applicability of different types of kinetic considerations to different reactor types is presented in Table 2.3, adapted from (Dunn, et al. 2003). This shows that in the case of continuous operation – like in the project – use of zero-order kinetics is acceptable, especially for primary estimations.

Table 2.3 Kinetics for various reactor types

Reaction Kinetics	**Batch Tank**	**Continuous Tanks-in-Series or Tubular**	**Continuous Single Tank**	**Fed Batch**
Zero order	OK	OK	OK	Low conversion only
First order	Best	Best	Low conversion only	Best
Substrate inhibition	Low initial concentration	Low tank concentrations	Best	Best
Product inhibition	Best	Best	Low conversion only	Low conversion only
Production triggered by shift in environment	OK for temperature shift	Possible	Not suitable	Best for concentration shift

2.5 Bioreactor design

2.5.1 Bioreactor design – basic guidelines

The bioreactor is a vessel in which the core biological reactions take place. In any process, whether chemical or biological, it plays a vital role. Any plant design, when all the upstream constrains were identified – in terms of bioprocesses, most of all cell factory operation characteristics and media construction and preparation – has to begin from a certain element, which puts the most influence on the rest of the operation processes – namely – the reactor. This fact is greatly emphasized when one looks at an onion model of process design, which is shown in Figure 2.12, adapted from (Smith, 2005).

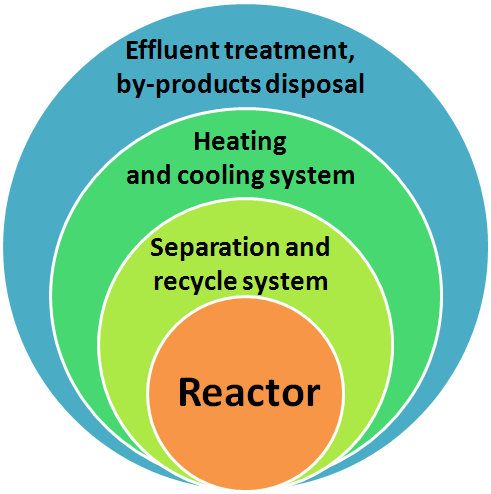


Figure 2.12 The onion model of process design

This simple graph shows a very important fact – the whole process has to be built up around one chosen core element – the reactor. Any new element incorporated into the plant has to be based on the previous elements, thus no step can be skipped and the order cannot be changed.

2.5.2 Reactor operation mode – batch vs. continuous

There are three main operation modes of the reactors: batch, fed batch and continuous. Each has some advantages and disadvantages which have to be carefully weighted according to the product formulation and culture used. Table 2.4, adapted from (Doran, 1995), shows general guidelines for the choice of a reactor's mode of operation.

Table 2.4 Advantages and disadvantages of reactor's modes of operation

Mode of operation	Advantages	Disadvantages
Batch	Equipment simple. Suitable for small production	Downtime for loading and cleaning. Reaction conditions change with time.
Continuous	Provides high production. Better product quality due to constant conditions. Good for kinetic studies	Requires flow control. Culture may be unstable over long periods.
Fed batch	Control of environmental conditions, e.g. substrate concentration	Requires feeding strategy to obtain desired concentrations.

2.5.3 Reactor control and operation

The reactor should maintain a favorable environment for the culture. In a perfect case scenario this can be brought down to uniformity and constancy of parameters such as:

- Temperature;
- Pressure;
- pH;
- mixing;
- shear stress;
- media composition.

Obviously, it is not possible to reach such a state in real big-scale applications. Although well-mixed conditions are not achievable, there still is a lot of control and measurement required just to run the process. Common operation variables, which have to be supervised in different types of reactors, are shown in Table 2.5, adapted from (Dunn, et al. 2003).

Table 2.5 Operation variables for different reactor types

Batch	Continuous	Semi-continuous
Initial medium composition and inoculums	Inlet medium composition	Feed and initial substrate composition
Temperature, pressure	Temperature, pressure	Temperature, pressure
pH if controlled	pH if controlled	pH if controlled
Reaction time	Liquid flow rate (residence time)	Liquid flow rate (residence time)
Aeration rate	Aeration rate	Feeding rate and control program
Stirring rate	Stirring rate	Aeration rate
		Stirring rate

2.5.4 Airlift bioreactors (ALR)

The reactor types working on the "airlift principle" can be divided into bubble columns and airlift reactors (airlifts, ALR). Mixing required in the bioreactor is achieved by the entrainment of liquid by the supplied gas bubbles, due to the buoyancy difference and return flow of the liquid to satisfy continuity, as the volumetric fluid flow rate is of much smaller magnitude than the gas flow (Deckwer, 1992). If the return flow is separated by some kind of a physical barrier, the reactor is categorized as an airlift.

Of the many kinds of bioreactors, ALRs have the fewest mechanical parts in the active area, while still maintaining a relatively low level of construction complexity. This is of great importance when mechanical wear and corrosion can be a risk. Another advantageous technical characteristic of the reactor is a high heat transfer rate, which enables the maintenance of a stable, uniform temperature profile throughout the reactor and allows for reactions with high enthalpy change. Furthermore, when a liquid-solid phase is present, which is the case in this project, relatively high rates of circulation allow reaching close to uniform solid phase distribution in the liquid, i.e. biomass (Deckwer, 1992). What is more, the cost of the reactor is moderate in comparison with others types, and scale-up, even to sizes as large as 200 m^3,is possible. Moreover O&M (operation and maintenance) costs, including energy use, can be greatly reduced when compared to mechanically-agitated types. All of the abovementioned advocate the further investigation of the airlift principle reactor type as the basic choice for the project, which is done in this chapter.

Figure 2.13, taken from (Merchuk, et. al, 1999), gives a comparison of the specific energy demand of different types of reactors as a function of provided oxygen transfer rates (OTR). It can be seen that for the same mass transfer efficiency (expressed as OTR) airlift designs can use up to 10 times less energy than the CSTR types.

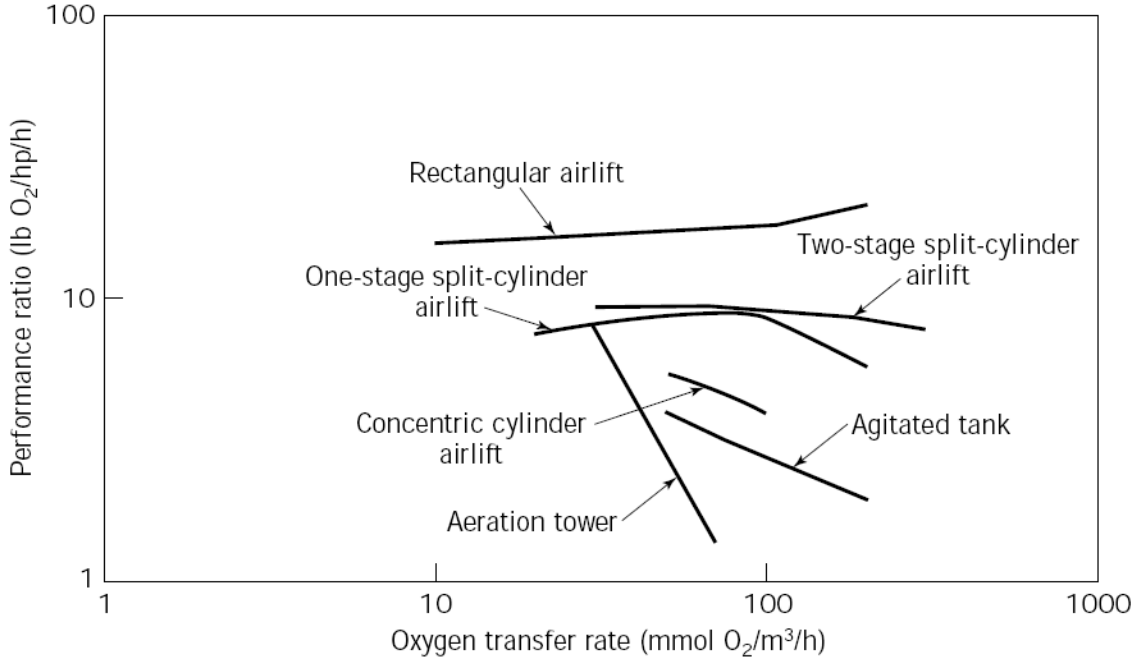


Figure 2.13 Oxygen transfer efficiency of different reactor types

Airlifts vs. bubble columns

Hiding behind the term airlift bioreactor is a wide range of pneumatic devices for contacting gas with a liquid (liquid-solid). Another distinct feature of these is that the fluid circulation is done in a defined pattern, inside separate channels for upflow (riser) and downflow (downcomer). The feed gas agitating the reactor is usually air or, less often, different gases. Apart from agitation, the construction and the gas flow facilitates mass transfer between the dispersion phases – either into or from the liquid phase (Merchuk and Gluz, 1999). The main difference between ALRs and bubble columns (which are also pneumatically agitated) lies in the type of fluid flow, which depends on the geometry of the system.

The bubble column is a simple vessel into which gas is injected, usually at the bottom, and random mixing is produced by the ascending bubbles (Jakobsen, 2008). On the contrary, in the ALR, the fluid circulation patterns are determined by the design of the reactor, primarily the closed loop created by the downcomer and riser.

The gas is usually injected near the bottom of the riser. The extent to which the gas disengages at the top, in the gas separator[1], is determined by the design of this section and the operating conditions. The gas fraction, which does not disengage but is entrapped by the descending liquid and taken into the downcomer, has a significant influence on the fluid dynamics in the reactor and hence on the overall reactor performance (Merchuk and Gluz, 1999).

[1] Also referred to later as the (reactor) headspace

2.5.5 Airlift reactor construction

There are two main groups of airlift reactors differing in the loop type. It can either be external (circulation takes place in separate channels) or internal (one of the channels is created by division of the reactor space by a barrier of some kind). Both types are presented in Figure 2.14 taken from (Merchuk and Gluz 1999).

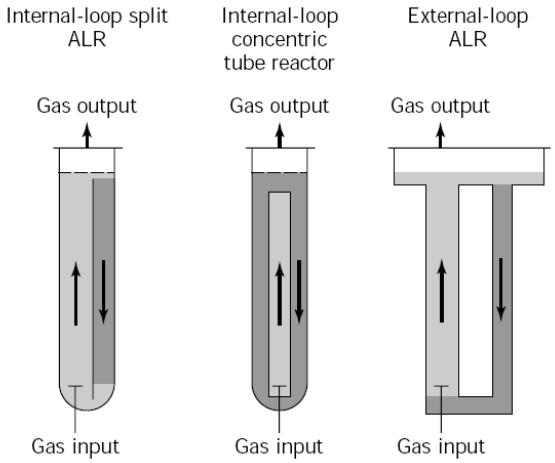


Figure 2.14 Main types of airlift reactors

The designs of both types can be modified further, leading to variations in the fluid dynamics, in the extent of bubble disengagement from the fluid, and in the flow rates of the various phases.

Regardless of the modifications to the basic construction, there are always four sections present:

• *Riser* – vertical, usually cylindrical part of the reactor where the gas is injected at the bottom and the upward dispersion flow prevails;

• *Downcomer* – parallel to the riser and connected to the riser both at the bottom and top; gas-liquid flow is predominantly downward. The circulation in the reactor is forced by the mean density difference between the fluid in this section and the riser;

• *Base* – section connecting the downcomer and the riser at the bottom of the ALR. Usually it is kept very simple, though there were reports that it can influence gas holdup, liquid velocity, and solid phase flow (Merchuk and Gluz, 1999), (Chisti, 1989);

• *Gas separator* – connects the riser to the downcomer at the top of the reactor. It is responsible for facilitation of liquid recirculation and separation of gas from the liquid phase. Proper design allows the control of gas content in the downcomer section (Merchuk and Gluz, 1999);

One should note that the characteristics of the transfer processes will differ between the sections, but the design of each section may have an impact on the performance and characteristics of other sections (Asenjo and Merchuk, 1995).

Advantages of Airlift Bioreactors

Even though the conventional, mechanically stirred reactors provide all the necessary requirements for microbial cultures, ALRs are still considered superior in most cases – primarily because of different fluid mechanics.

In conventional reactors the mixing is done by the mechanical stirrer. In its vicinity the shear forces – and energy dissipation – are the greatest, producing one order of magnitude discrepancy between the average shear gradient and the one in the stirrer surroundings. As all the transport phenomena are interlinked, undesirable non-uniform gradient fields are created for all the crucial parameters, such as shear stress, temperature, concentration etc.

In ALRs, the gas is also injected at a single point, but the mixing occurs primarily due to the density difference of the fluids in the downcomer and riser parts, producing a pressure difference at the bottom, which drives the circulation. Thus, the direct contribution to dynamics of the system, for ALRs, is small (Merchuk and Gluz, 1999), removing a vast majority of the problems connected with the locus-like mixing energy and shear introduction.

Therefore, the main advantage of ALRs is homogeneity of the of stress forces, which is especially important for shear-sensitive cultures (Merchuk and Gluz, 1999). Other advantageous features include:

- Mechanical simplicity of the reactor (no shaft and shaft seals, which pose a contamination risk);
- Higher energy efficiency (important for low-value products, as energy use can have a significant input into the final cost of the process);
- Higher mass transfer rates (compared to mechanically stirred reactors);
- Higher flexibility (lower performance changes in case of changes in operating conditions);
- Space for improvements in energy demand, mass transfer characteristics etc. (by i.e. double-sparger or deep shaft construction).

There is however one big disadvantage of the airlift reactors – minimum volume requirements for proper operation (Merchuk and Gluz, 1999).

2.5.6 Airlift design

When considering the ALR design, several main variables should be considered. Most of them, unfortunately, are interlinked and influence each other. In most cases, the theoretical estimations are inaccurate or impossible. Therefore, most data on parameters important for airlift design, given below, must come from simulations or research conducted in similar projects.

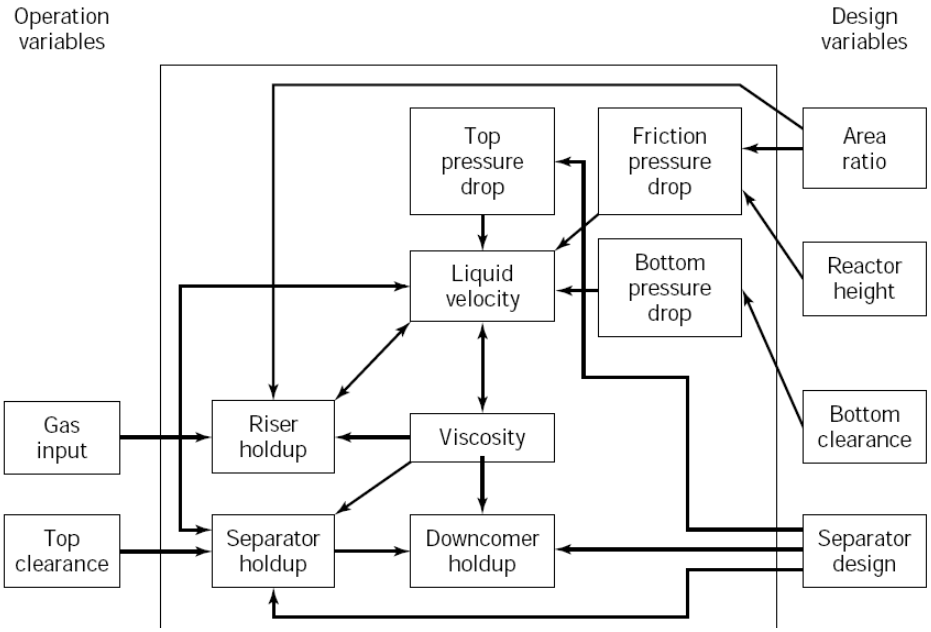


Figure 2.15 Design variables' interaction in ALR design

Viscosity, not included in Figure 2.15 taken from (Merchuk and Gluz, 1999), is also an important parameter, yet it mainly depends on the gas holdup and liquid velocity and it will probably change over time in the actual process. Nevertheless, it is clearly visible that the reactor operation, after the design phase, depends basically only on one externally controlled parameter – gas input. That fact puts additional pressure on proper initial design, as usually the feed rate is somehow fixed (like in the case of the project), further limiting potential modifications to the process when the plant has already been built.

2.5.7 Biofilters

All the biofilter-type plants have been found to be very successful in waste gas cleaning operations (Friederich and Werner, 1999). However they are not directly applicable for the case of the project (see section 4.5.2). Yet, because of their potential in hydrogen sulfide remediation, basic types are briefly introduced.

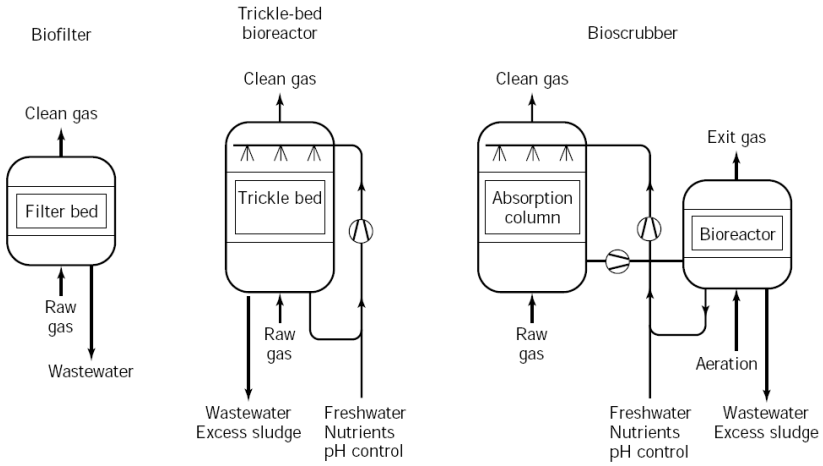


Figure 2.16 Reactors for waste gas treatment

Figure 2.16, taken from (Friederich, et al., 1999), shows three basic types of reactors found in typical biological gas-cleanup plants. Most of them work with a packed bed, through which the gas stream is passed. The system can be very efficient in operation (Tang, Baskaran and Nemati, 2008) since the liquid, which has the objective of dissolution of gaseous components, usually has pH over neutral. It greatly facilitates mass transfer of sour gases – such as hydrogen sulfide – into the solvent because H_2S present in the liquid is quickly converted into its ionic species. However, the biological clean-up technologies are still rather reserved for low pollutant concentrations even though in most cases they are less costly than the chemical means (Friederich and Werner, 1999).

3 FLUID MECHANICS AND TRANSPORT PROCESSES FOR BIOPROCESS DESIGN

3.1 Transport phenomena

Most transport processes occur at the interfaces, in the boundary layers or their vicinity. Their character is governed by a system of nonlinear, mainly partial, differential equations. The data needed for tackling transport processes involves not only the fluid field, but also the gradients of velocity, temperature and concentrations with boundary conditions set on them. The analytical solutions exist only for the most basic geometries – they are hardly ever applied in the actual industrial practice. Numerical calculations can be made, but still, for more complex (namely turbulent) flows getting a reliable result can be either very costly or impossible. Nevertheless, for practical applications there are some simplified approaches, based mainly on experimental data, allowing the determination of some of the most crucial parameters like friction losses in the hydraulic systems or mass and heat transfer in industrial practice. The most common engineering approaches to transport processes involve the use of so-called transport coefficients and dimensionless numbers. In this chapter, the theory behind the transport processes playing the most prominent role in the bioprocess design shall be discussed.

Analogies in momentum, heat and mass transfer

All the above mentioned processes are said to be very similar, which can be easily seen after writing the most basic flux equations:

$$\tau' = \mu \frac{\partial u}{\partial y} \qquad \dot{q} = -k \frac{\partial T}{\partial y} \qquad j_A = -D_A \frac{\partial \rho_A}{\partial y}$$

Thanks to that, results obtained from research of one type of the process can be applied to the others when certain conditions are fulfilled. Here the discrepancies occur, as different set of conditions have to be satisfied for all the types of transport phenomena.

Momentum transfer is one of the major fields in fluid dynamics. Similarity is sustained when geometry and flow characteristics are alike and the boundary conditions are in correlation. Heat transfer requires all of the above with the additional need for analogy of the temperature field. Mass transfer – probably the most complex, apart from the aforementioned – requires corresponding concentration profiles to fulfill the similarity conditions.

3.2 Mass transfer

Mass transfer phenomena have an impact on all facets of bioprocessing. Transport intensity often determines the bioreactor's productivity and downstream operations. Gas–liquid mass transfer problems usually arise during the supply of oxygen from a gas phase to liquid culture and during removal of metabolic carbon dioxide from the culture fluid. Also, for not so common gaseous fermentations, the issue of sufficient introduction of feed into the fermentation broth is one of the limiting steps in such process development. Similarly, mass transfer has to be tackled again during recovery operations, i.e. distillation.

Liquid–liquid mass transfer occurs when oxygen is supplied through liquid carriers such as perfluorocarbons, during liquid–liquid extraction and during degradation of water-immiscible liquid substrates. Solid–liquid mass transfer problems are common during recovery by adsorption, chromatographic separations and in operations such as crystallization (Chisti, 1999). The performance of solid-phase biocatalysts such as immobilized cells and enzymes is often limited by solid–liquid mass transfer. Solid–liquid mass transfer effects influence the work of membrane separations such as micro- and ultrafiltration. Transport within solid particles or intra-particle mass transfer becomes limiting in certain cases. Gas–solid transport can be seen during some drying situations (Hauke, 2008).

Finally, the transport of a solute through any fluid or space is governed by the molecular diffusivity or the diffusion coefficient of the solute in the fluid or solution.

As the project involves gaseous substrates and the fermentation will be of submerged type, the main focus will be on gas-liquid transfer, which is discussed in more detail in this chapter.

3.2.1 Diffusion and Fick's law

Diffusion is the transport of a species due to concentration gradient in a mixture.

The law that governs the process states that the diffusive flux of matter is related to and forced by the non-uniform concentration field. The formula given by Fick is as follows:

$$j = -D\nabla c$$

where j [mol/L^{2}T] denotes molar flux. For transient phenomena a second law was established:

$$\frac{\partial c}{\partial t} = \nabla(D\nabla c)$$

Where c is concentration of solute and D [L^2/T] diffusivity.

3.2.2 Diffusion coefficient

The diffusion coefficient reflects the underlying characteristics of the molecules in the mixture and is related to the product of the mean velocity of the molecules and the average distance between molecular interactions (Nellis and Klein, 2008). However, that is not much help in engineering practice. More direct relations to typical physical system parameters are necessary. Methods of diffusivity estimation for gas-liquid diffusion will be provided in the chapter.

The diffusion coefficient is a transport property representing the ability of species (solute) to diffuse through a medium (solvent). Diffusivity depends on temperature, the type of solvent and its viscosity, and the concentration of solute in solution. Diffusion coefficients in liquids and gases generally increase with temperature. Liquid-phase diffusivities are little affected by pressure; but in gases, diffusivities decline as pressure increases.

Table 3.1 Diffusivities of some common solutes in diluted liquids (Chisti, 1999)

Solute	**Solvent**	**Temperature [°C]**	**D_L [$\times 10^9$ m^2 s^{-1}]**
CO_2	Water	20	1.50
CO_2	Water	25	2.00
Ethanol	Water	25	1.24
Glucose	Water	20	0.60
Oxygen	Water	20	1.80
Oxygen	Water	25	2.41
Water	Ethanol	25	1.13

When diffusion coefficients are not available they can be estimated, yet there is no simple theory behind it. Typical simplifying assumptions are that the solution is infinite and the mixture ideal and binary. For such cases, under molar volumes of solutes < 0.5 m^3/kmol, Wilke-Chang[2] equation can be used:

$$D_L = 1.173 \times 10^{-16} \frac{(\chi M_L)^{0.5} T}{\mu_L V_M^{0.6}}$$

Association parameters (χ) of some common solvents can be found in the literature, i.e. (Chisti, 1999). For water as a solvent, the association parameter is taken as 2.6. Molecular volumes of simple substances (V_M) are given in Table 3.2.

[2] where M and μ are the molecular weight and the viscosity of the solvent, respectively; T is the absolute temperature, V is the molar volume of the solute at its boiling point, and χ is the association parameter, a measure of polar interactions among molecules, of the solvent (Chisti, 1999).

Table 3.2 Molecular volumes of chosen simple substances (Hendricks, 2006)

Substance	V(solute) [cm^3/gmol]
H_2	14.3
O_2	25.6
N_2	31.2
CO_2	34.0
NH_3	25.8
H_2O	18.9
H_2S	32.9

3.2.3 Film theory and mass transfer coefficients

It is assumed that the fluid is stagnant and the process is steady-state. The interface is surrounded on both sides by two very thin boundary layers in which transport can occur only by means of diffusion. The film theory states that the intensity of mass transfer depends on the resistance, which films on the both sides of the interface pose for the process. Figure 3.1, taken from (Chisti, 1999), illustrates the case for the gas-liquid phase boundary.

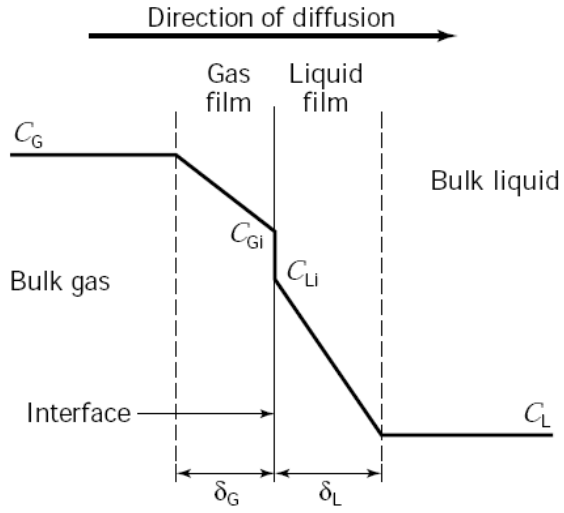


Figure 3.1 Steady-state concentration profile around gas-liquid interface

Now, the flux related to transport (J) of the diffusing species can be related to the concentration gradient (ΔC) in the film and to the film thickness (δ) as follows:

$$J = \frac{D}{\delta}(\Delta C)$$

The ratio of D/ρ is usually referred to as the mass transfer coefficient and denoted as k.

For a steady state system the fluxes balance out, giving the set of equations[3]:

$$J = k_G(C_G - C_{Gi}) = k_L(C_L - C_{Li})$$

Thus, the overall mass flux from the gas to the liquid phase may be written as:

$$J = k_L(C^* - C_L)$$

The saturation concentration C* in the liquid is related to the gas phase concentration of the diffusing component by Henry's law:

$$C_G = HC^*$$

H being the dimensionless Henry's constant. Finally, the overall mass transfer coefficient (into the liquid phase) can be expressed in terms of film resistances:

$$\frac{1}{K_L} = \frac{1}{k_L} + \frac{1}{Hk_G}$$

This allows determining a very important fact – namely – which side is the limiting one. The phase which has greater influence on the interfacial transfer can be decided using Table 3.3, which is based on (Hendricks, 2006).

Table 3.3 Interface resistance significance

H	**Solubility**	**Gradient**		**K_g**	**K_L**
		Aqueous Phase	**Gas Phase**		
Large	Low	Steep	Shallow	>0	$\approx k_L$
Small	High	Shallow	High	$\approx k_g$	>0

Knowing that the diffusivities in the gases are usually three to four orders of magnitude bigger than for liquids, for sparingly soluble gases (like oxygen in water), the overall mass transfer can be approximated by $k_L$only. For that reason it is a common practice to express it as $k_L a_L$ [1/T].

3.2.4 Oxygen mass transfer coefficient

The driving force behind the mass transfer is usually easy to determine and depends mainly on temperature and pressure. The $k_L a_L$, however, is heavily dependent on fluid and flow properties as well as bioreactor configuration. This translates into a long list of reactor operating parameters having influence on the value of the coefficient, presented after (Chisti, 1999) in

Table 3.4.

[3] Subscripts L and G are for liquid and gas respectively; superscript * used for saturation values.

Table 3.4 Factors influencing gas-liquid mass transfer in bioprocesses

Temperature	Flow parameters of non-Newtonian fluids
Pressure	Presence of surfactants and ions
Diffusivity	Concentration of solids
Viscosity	Hydrophobicity of solids
Density	Morphology of solids
pH	Shear rate or power input
Ionic strength	Geometry of the bioreactor
Surface tension	

Estimation of the actual value of k_La_L is one of the crucial steps in bioprocess design – oxygen limitation being the most common culprit. Because of that, most measurements and predictions are based on results obtained for oxygen.

Table 3.5 Typical values of overall mass transfer coefficient in bioprocesses

Process	**k_La_L [s^{-1}]**
Fungal fermentations	10^{-2}
Bacterial and yeast fermentations	10^{-1}
Wastewater treatment	3×10^{-3}

3.2.5 Mass transfer coefficient for gases different than oxygen

When a gas different than oxygen is to be fed into the binary mixture, the values of the overall mass transfer coefficient can in some cases be approximated using following equation (Chisti, 1999):

$$(k_L a_L)_{gas} = \frac{D_{gas}}{D_{oxygen}} (k_L a_L)_{oxygen}$$

3.2.6 Multi-component mass transfer

When there is more than one gaseous and one liquid species present in the media subjected to diffusive mass transfer, the most simple form of Fick's law does not apply. It is possible to use either a generalized or matrix form of Fick's law, yet the diffusion coefficients in the equation no longer maintain their physical meaning and have to be experimentally obtained (Taylor and Krishna, 1993). Certain methods for dealing with such problems were discussed in (Rousseau, 1987) and (Cussler, 1997).

One should also mention that other effects such as ionic strength or interactions between species can have strong influence on the overall transfer rates (Taylor and Krishna, 1993).

An example relevant to the project is given in (Kohl and Nielsen, 1997), when the carbon dioxide presence at high concentrations hinders mass transport of hydrogen sulfide.

3.3 Fluid mechanics

Fluid mechanics covers a wide area of problems connected with fluid flows. The cases of multiphase flows are usually even more problematic than the ones typically encountered. The treatment of fluid mechanics-related topics in literature is common for CSTR systems, but not for airlifts. Because of the lack of a sufficient amount of data and reasons mentioned in Chapter 2.3, only a couple of concepts will be introduced in the section – rather as examples than any kind of introduction.

3.3.1 Flow regime

The general multiphase flow pattern in bubble columns is usually one of the three types depicted below in Figure 3.2 as in (Deckwer, 1992):

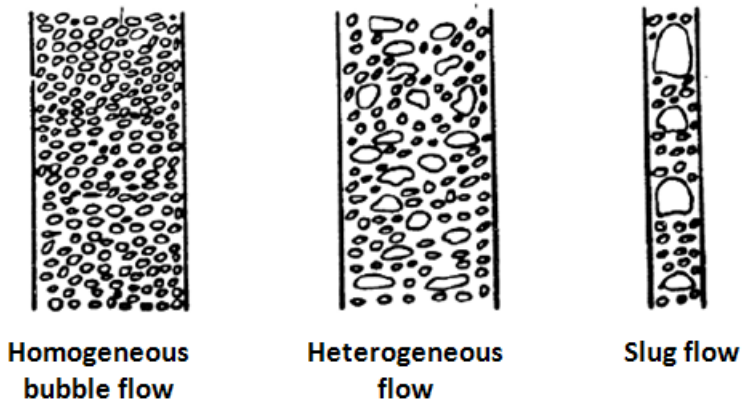


Figure 3.2 Flow regimes common for bubble columns

Although the actual flow can be very different, depending mainly on superficial gas velocity, feed rates and system configuration (Jakobsen, 2008). Determination of a typical pattern for airlifts can be done using Figure 3.3, taken from (Merchuk and Gluz, 1999).

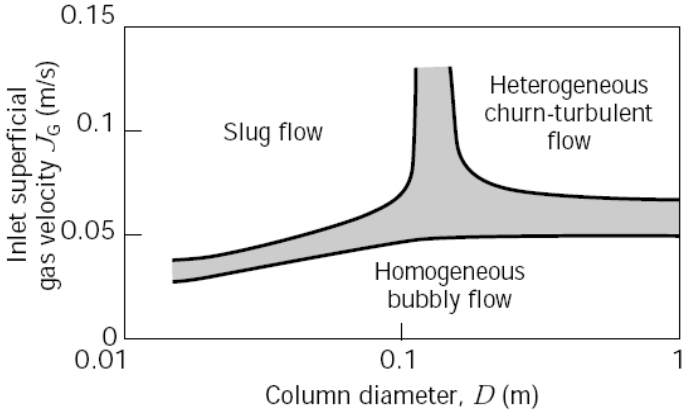


Figure 3.3 Three most common flow regimes in airlift reactors

The rule of thumb says that one should avoid operation in the slug flow region. Homogeneous bubbly flow is also not always desirable. More detailed discussion can be found in (Chisti, 1989), (Asenjo and Merchuk 1995), (Chisti, 1999).

3.3.2 Power law and its significance in bioprocessing

Viscosity, seemingly unimportant, is a very important characteristic of fermentation fluids. Not only does it have influence on the flow (included in Re number), but also on downstream processing (approach to separation) and the reactor operation (power demand, mixing behavior). For that reason a basic introduction to the subject is given further in the text.

Power law and apparent viscosity

Consider the situation depicted below, taking: steady state conditions, laminar flow and incompressible fluid.

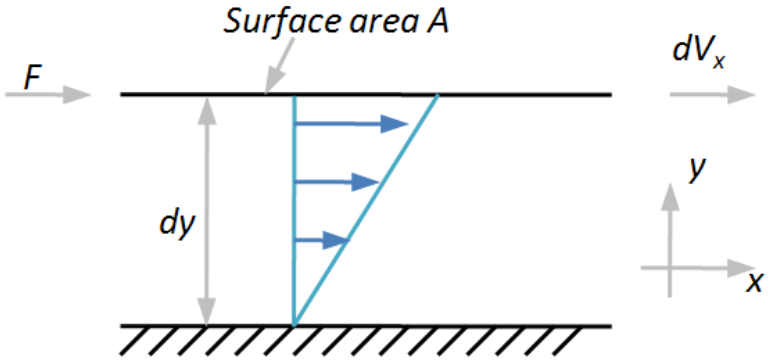


Figure 3.4 Unidirectional shear flow representation (Chhabra and Richardson, 2008)

The process can be described by the equation:

$$\frac{F}{A} = \tau_{xy} = \mu(-\frac{dV_x}{dy}) = \mu\dot{\gamma}_{xy}$$

In other terms, the shear rate is directly proportional to the shear stress. The proportionality constant in the formula is a property of fluid called viscosity. In this case, it does not depend on any other system parameters and the fluid is called a Newtonian fluid.

Power law

In real systems viscosity is not constant. It changes with parameters such as temperature, but also for most working fluids with the shear stress to which the fluid is subjected.

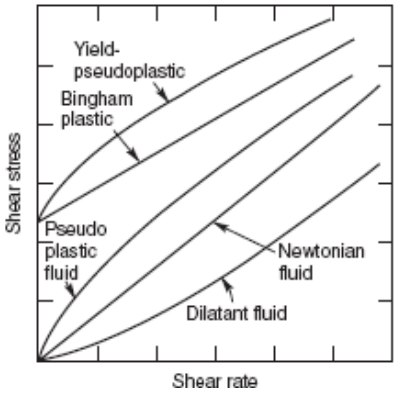


Figure 3.5 Most common non-Newtonian flow behavior as in (Chhabra, et. al, 2008)

The commonly used equation describing the behavior of non-Newtonian fluids, given below, is called the power law.

$$\tau = m\dot{\gamma}^{n}$$

Now, one can define apparent viscosity as:

$$\mu = m\left(\frac{\tau}{\dot{\gamma}}\right)^{n-1}$$

(m is the fluid consistency coefficient and n, the flow behavior index; both are empirical)

Depending on the exponent, the fluids can be divided into three groups:

- n<1, shear-thinning;
- n=1, Newtonian;
- n>1, shear thickening.

The most common non-Newtonian fluids are the shear-thinning ones. Figure 3.6 shows that the behavior of such a liquid is based on (Chhabra and Richardson, 2008). More detailed information can also be found in the book.

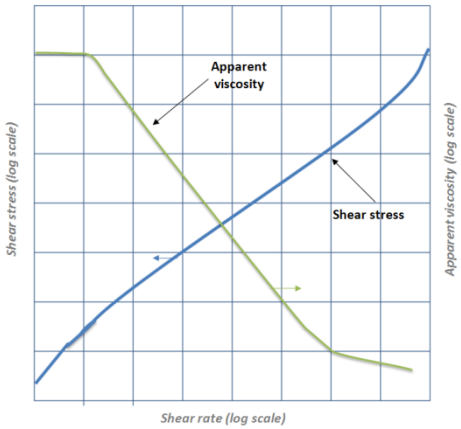


Figure 3.6 Representation of shear-thinning behavior

One can expect that the fermentation broth in the project will be of this type.

3.3.3 Aeration – bubbles and particles in a fluid; dispersions

When a particle is introduced into to the fluid it becomes subjected to the forces present within. Because of this it accelerates; yet usually in a short period of time it reaches its terminal velocity when the gravitational, buoyancy forces and fluid dynamic drag balance out. It is of crucial importance to know the particle's terminal velocity both when trying to quantify particle settling and bubble rise.

For a particle gravity settling in a power-law liquid, at Re < 1, the terminal velocity can be approximated with the formula:

$$V = \left[\frac{g \cdot d^{n+1} \cdot (\rho_s - \rho)}{18 m X(n)} \right]^{1/n}$$

The expression for X(n) can be found in (Chhabra and Richardson, 2008).

Bubbles

Usually the most daunting problem in gas-liquid reactor design is the mass transfer. Gas is introduced and bubbles are created producing multi-phase flow of various regimes in the reactor. Description of bubble behavior after initial introduction into the system is a very difficult task, yet parameters such as bubble superficial velocity, gas holdup, gas holdup time, bubble terminal velocity and mean bubble diameter have to be evaluated to properly design and run a process. A very detailed study of the issue for airlift reactors is made in (Merchuk and Gluz, 1999) as well as (Asenjo and Merchuk, 1995).

Bubble size

In practice, the spargers introducing the gas into the reactor give bubbles of approximately 3 mm in diameter. Going below 1 mm is usually avoided because of problems in operation (Doran, 1995). On the other hand, bubbles bigger than 6 mm have a strong tendency to coalesce, hindering mass transport (decreased interfacial area) and flow regime in the reactor.

An important fact in gas disengagement is critical bubble radius, as the terminal bubble rise velocity is not a continuous function of bubble size. Transition over the critical value results in a 6- to 10- fold increase in the terminal velocity (Chhabra and Richardson, 2008). Also surprising is that for free rise and power-law fluids, the boundary radius does not depend much on the fluid properties and character and can be estimated using the formula:

$$r = \sqrt{\frac{\sigma}{g(\rho_l - \rho_s)}}$$

Discussion on behavior of bubbles in dispersion can be found in (Chhabra and Richardson, 2008) and (Chisti, Mass transfer, 1999). The latter also provides a very miniscule description of aeration and gas-liquid mechanics specifically for ALR design. More information on the topic can also be found in (Asenjo and Merchuk, 1995) and (Merchuk and Gluz, 1999). For a more common approach, especially for CSTRs, one can refer to any of the following: (Doran, 1995), (Dunn, et al. 2003), (Nielsen, Villadsen and Liden, 2003).

3.3.4 Dimensionless numbers

Buckingham ∏ theory

Assume that a phenomenon is governed by N different variables. If the variables have a physical meaning, they will be quantified using M fundamental quantities (i.e. length, time, mass, etc.). The ∏ theorem states that the process can be described using (N-M) equations involving dimensionless ∏ groups.

If one would compare two systems characterized by the same dimensionless groups, and their values would be equal, it can be assumed that they are similar.

Dimensionless numbers

The ∏ theorem creates a basis for the construction of a dimensionless description of a process. It does not, however, put any emphasis on the physical significance of the groups. In engineering practice, there are several dimensionless numbers in use which describe relevance on different physical phenomena and properties, allowing better analysis of the processes on a common basis, as well as appliance of results to different scales and conditions.

As stated by (Chisti, Mass transfer, 1999), mass transfer in bioreactors is generally influenced by:

- Mass transfer coefficient;
- Diffusivity;
- Fluid density;
- Viscosity;
- Characteristic length;
- Velocity of the flow;
- Gravitational acceleration and density difference (for natural convection systems).

All of the above properties can be grouped into dimensionless groups most commonly used in the description of mass transfer processes:

- *Re (Reynolds number)* = $\frac{\text{Inertial force}}{\text{Viscous force}} = \frac{\rho_L U_L d}{\mu_L}$
- *Sh (Sherwood number)*= $\frac{\text{Total mass transfer}}{\text{Diffusive mass transfer}} = \frac{k_L d}{D_L}$
- *Sc (Schmidt number)* = $\frac{\text{Momentum diffusivity}}{\text{Mass diffusivity}} = \frac{\mu_L}{\rho_L D_L}$
- *Gr (Grashof number)* = $\frac{(\text{Inertial force})(\text{bouyancy force})}{(\text{Viscous force})^2} = \frac{d^3 \rho_L \Delta\rho g}{\mu_L^2 D_L}$

- *Fr (Froude number)* = $\frac{\text{Inertial force}}{\text{Gravitation force}} = \frac{U_L^2}{gd}$

Particular numbers are used depending on the importance of the ratio of different phenomena. Therefore, respective numbers should be considered under the circumstances presented in Table 3.6.

Table 3.6 Cardinal dimensionless groups in mass transfer description

Number	**Situation in which it plays a role**
Re	Defines flow character and forced convection importance on transport phenomena
Fr	High density fluids and gravity-influenced flows
Sc	Determination of major diffusion mechanism
Sh	Importance of diffusive mass transfer
Gr	Buoyancy-driven natural convection in relevance to forced one

Typically all the empirical correlations and equations regarding mass transfer are given together with ranges of a respective dimensionless group in which they can be applied.

Alternative formulations of the given cardinal numbers and some of the less common ones can be found in the Appendixes.

4 THE GEOGAS PROJECT[4] – BIOPROCESS SCALE-UP CASE STUDY

The project is currently in the stage of shift from lab into pilot plant scale. To ensure proper implementation, certain assessments should be made. The analysis of available data and pointing out possible issues and solutions will be the subject of this chapter[5].

4.1 Lab scale experiment

When the project began several aims were set (Prokatin ehf., 2008):

- Utilize/clean exhaust gas from geothermal power plants;
- Develop microbial production system;
- Produce SCP;
- Produce enzymes and specialty chemicals.

Iceland has an extensive fishing fleet, but also a number of fish farms for which the produced SCP could become an additive for the feed. An economic study (Prokaria, VGK, 2005) showed positive results at current market prices. Abundant geothermal sites provide cheap steam and power, but also significant amounts of hydrogen, which could be used as a good energy source for fermentations. Also, there should be no problem with water availability for cooling and the process itself. However, to move into more sophisticated product formulation, at least a pilot plant would be necessary. Having known that, a place for the lab scale project was chosen at the Nesjavellir power plant shown below.

Figure 4.1 The Nesjavellir power plant

[4] All materials and data in this chapter regarding the GEOGAS project were provided and are the property of Prokatin ehf.

[5] As a site, the Nesjavellir power plant will be analyzed and data provided by Prokatin ehf. will be taken as assumptions. Installation (bioprocess) up-time will be taken as 8760 hr yr^{-1} for simplifying purposes.

In order to obtain data on the culture, the Laboratory of Geothermal Biotechnology was established in the power plant. Pictures below illustrate the laboratory experiment setting.

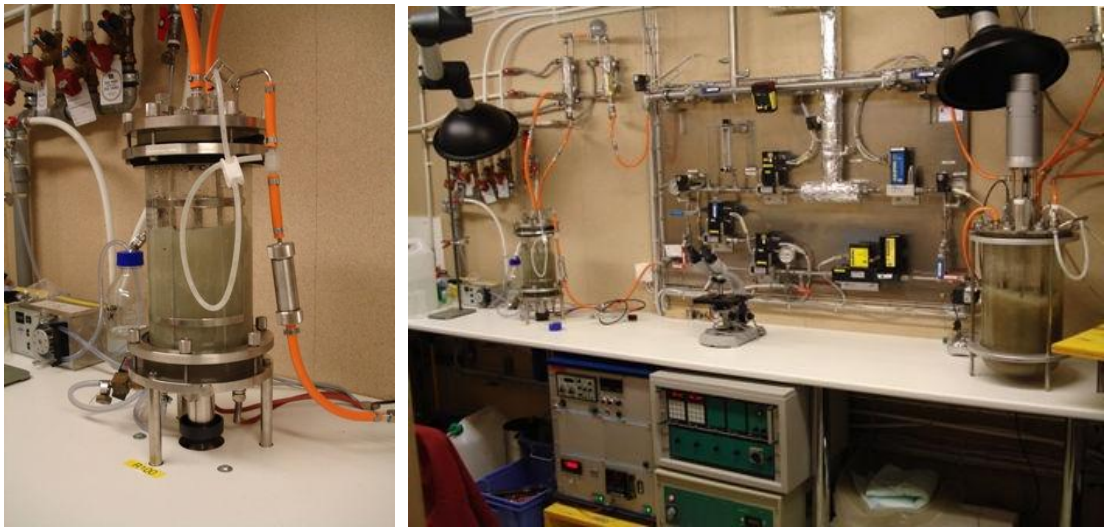


Figure 4.2 Lab scale set-up (Copyright, Prokatin ehf., 2008)

4.2 Cell factory[6]

Extensive data on the culture used in the process is not only important for running the process in a stable way, but also, in the further process development and intensification, accounts for most of the yield and production increases. A short description of the microbes in use will be made in this sub-chapter.

4.2.1 Strain selection

The culture investigated in the lab scale experiment was not isolated for a particular strain. From a site with similar conditions to that assumed for the project, samples were taken and grown selectively. The mixed culture was identified to be acido- and thermophilic, capable of running on a chemoautotrophic basis under aerobic conditions. It was determined that it can oxidize hydrogen sulfide, hydrogen and elemental sulfur as the energy source, whereas carbon is obtained through CO_2 fixation. Most probably depending on conditions, one of the strains in the culture becomes dominant. All the uptake of the feed is coupled with energy metabolism. H_2S is an electron donor preferred to hydrogen, however each of the strains present seem to prefer and do mainly one type (or step) of oxidation (Ævarsson, 2008). Under H_2S limitation the culture oxidizes the elemental sulfur, from the hydrogen sulfide oxidation, further into sulfuric acid. Severe energy source depletion makes bacteria go into a dormant state without damage to the culture if advantageous conditions are restored. The microbes show no signs of vulnerability to shear stress under well-mixed CSTR conditions.

The aforementioned factors would indicate bacteria of a colorless sulfur bacteria group most likely from genus *Thiobacillus* or *Acidithiobacillus*.

[6] In this chapter onwards, all the calculations are burdened with quite significant inaccuracy. Therefore the deviation from the 3-significant-digits convention use as well as rounding and approximation of results will not be justified and made according to author's own practice.

Over the period of lab experiments, the culture did not show signs of instability, which is especially important as the mode of reactor operation was chosen as continuous.

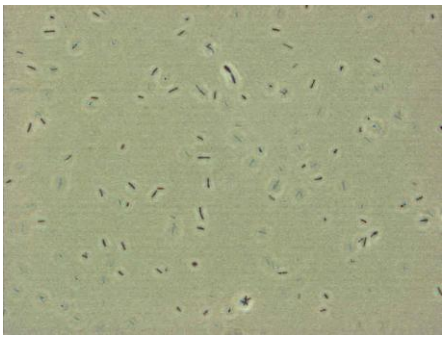


Figure 4.3 Culture under the microscope (Copyright, Prokatin ehf., 2008)

4.3 Preliminary material balances and flows

4.3.1 Main feed components – geothermal gas

Feed gas

As previously stated, there are three main components of the feed gas – CO_2, H_2S and H_2. Oxygen will also be fed to the reactor which, in total, gives four-component gas phase in the reactor.

For further calculations it will be assumed that the total discharged NCG input for the bioprocess will be at standard conditions and mass flow given below[7]:

Table 4.1 Emissions from Nesjavellir plant

Nesjavellir		
		unit/yr
CO_2	25000	Tons
H_2S	7500	Tons
H_2	400	Tons

The ideal gas law[8] for the mixture, integer molar weights of the components and individual gas constants from Table 4.2 are assumed.

Table 4.2 Individual gas constants of the feed gas components

Individual gas constant		
R_{CO2}	0.189	kJ/kgK
R_{H2S}	0.245	kJ/kgK
R_{H2}	4.157	kJ/kgK
R*	249.9	J/kgK

[7] Nitrogen and other gases' content will be neglected

[8] Justified for engineering purposes under low pressures

Resulting set of equations:

$$R^* = R_{mixture} = \sum \frac{R_i m_i}{m_i}$$ (individual gas constant for the mixture)

$$pV = (m^*)(R^*)T$$ (ideal gas law for the mixture)

yields volumetric flow of the gases and their respective shares on volume basis:

Table 4.3 Gas flows from the power plant

Volumetric flow			***Share***	
V_{H2S}	5,30 E+06	m^3/yr	22,3%	v/v
V_{CO2}	13,70 E+06	m^3/yr	57,5%	v/v
V_{H2}	4,81 E+06	m^3/yr	20,2%	v/v
V^*	23,80 E+06	m^3/yr	100,0%	total

4.3.2 Main product - SCP

It was assumed that yearly production of the plant will be 2500t of SCP, which will produce the revenue. For given typical biomass composition (Nielsen, et al., 2003), (Doran, 1995), $CH_{1.8}O_{0.5}N_{0.25}$ and average 7% (wt.) of mineral matter in the cells, one obtains requirements for the provision of all the basic elements into the system and molar mass of the organic matter.

Table 4.4 Biomass elemental composition

Element		**Molar wt.**	**wt. %**	
C	1.0	12.0	48.8%	wt./wt.
H	1.8	1.8	7.3%	wt./wt.
O	0.5	8.0	32.5%	wt./wt.
N	0.2	2.8	11.4%	wt./wt.
	Biomass	24.6	100.0%	g/mol

Table 4.5 Elemental requirements for production of 2500t of SCP

Element	**Molar wt.**	**mass**	
C		1134.1	t
H		170.1	t
O		756.1	t
N		264.6	t
nutrients (7% wt.)		175.0	t

It can be seen that the on-site resources can fulfill the demands of the given bio-protein production (see Table 4.1). Also, amounts of nitrogen (supplied as the nitrogen source) and nutrients (found in the biomass as mineral matter) can be estimated, as they influence the final economic balance of the plant.

4.3.3 Growth parameters

Were steady-state conditions and exponential growth to be maintained in the reactor, one can calculate the required specific growth rate (and doubling time). For simplified calculations and according to section 2.4.3, zero-order kinetics were used.

$$\mu = \frac{yearly\_production}{8760\frac{hr}{yr} \cdot X \cdot V_{react}} \left[\frac{kg\_BM}{kg\_BM \cdot hr} \right] = 0.114 \left[1/hr \right]$$

$$t\_doubling = \frac{0.693}{\ln(\mu + 1)} hr = 6hr\ 25\text{min}$$

4.4 Gaseous feed

Vapors can be liquefied through state change over the dew point. For some gases, it is impossible to make them liquid in such a manner, when only conditions close to ambient (standard or normal) are considered. In the gas stream rejected from the geothermal plants, one can assume that the NCG's share is of around 2%; although the actual amount and composition is completely dependent on the site and reservoir that are in use (DiPippo, 2007). When geothermal high temperature areas (common in Iceland) are considered, the main constituents of the NCG are carbon dioxide and hydrogen sulfide. What is quite unique for Iceland, in this case, is a relatively high share of hydrogen gas in the non-condensable part. Thus, for the gas feed, three components – namely CO_2, H_2S and H_2 – should be considered.

One of the key assumptions of the project was to utilize the gas as it was received from the power plant. As the gaseous components are both the carbon and energy sources, determining their behavior under the most likely bioreactor conditions is of crucial importance and will be investigated more thoroughly in the following sections.

4.4.1 Solubility of gaseous feed components - approximations

In general, the solubility of gases in water decreases with temperature. Taking into account the relatively high temperature of the process (taken as 50 ˚C) and the fact that the feed has to get from the gas phase into the vicinity of the bacterial cells, mass transfer and solubility are of crucial importance for successful operation of the plant.

Carbon dioxide and hydrogen sulfide, when in water, can dissociate showing acidic character and lowering system pH. pH, as a reactor parameter, is also important for the species distribution of the ions and bacterial metabolism, which may affect microbial growth.

Data obtained from (Engineering Toolbox, 2005) presented in Figure 4.4 shows temperature-dependent solubility profiles of gaseous constituents of the system.

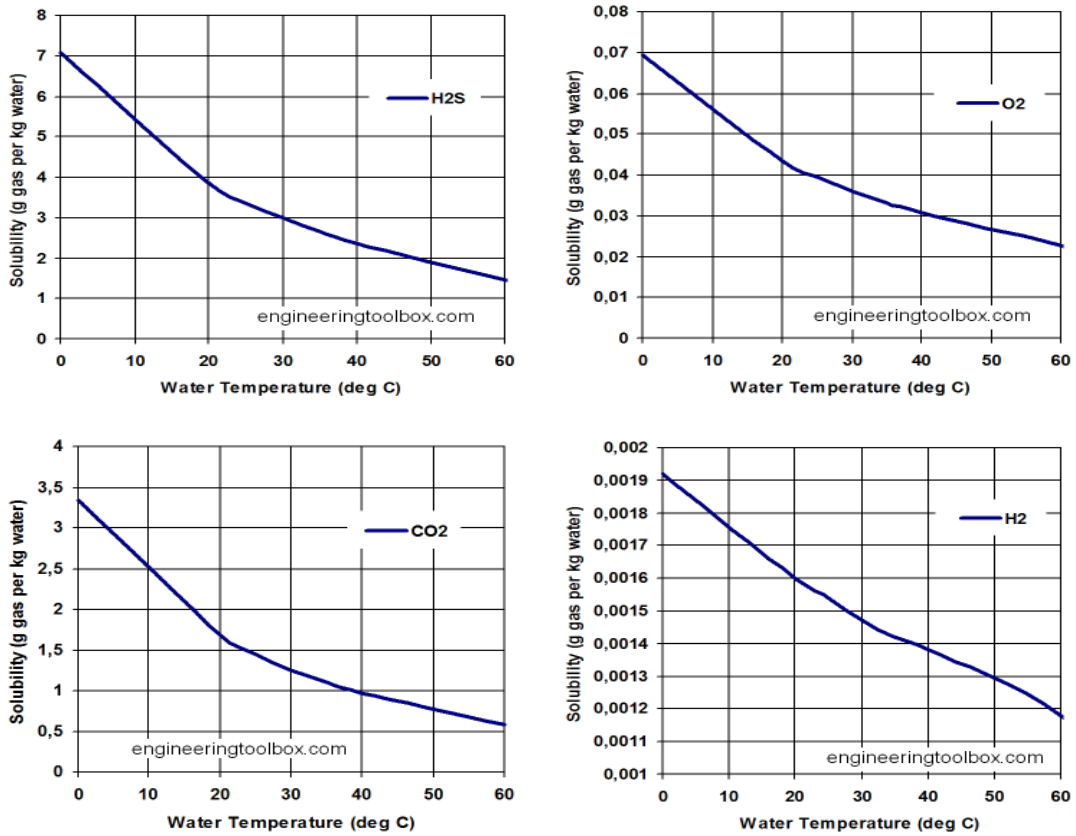


Figure 4.4 Solubility of gas feed components as a function of temperature

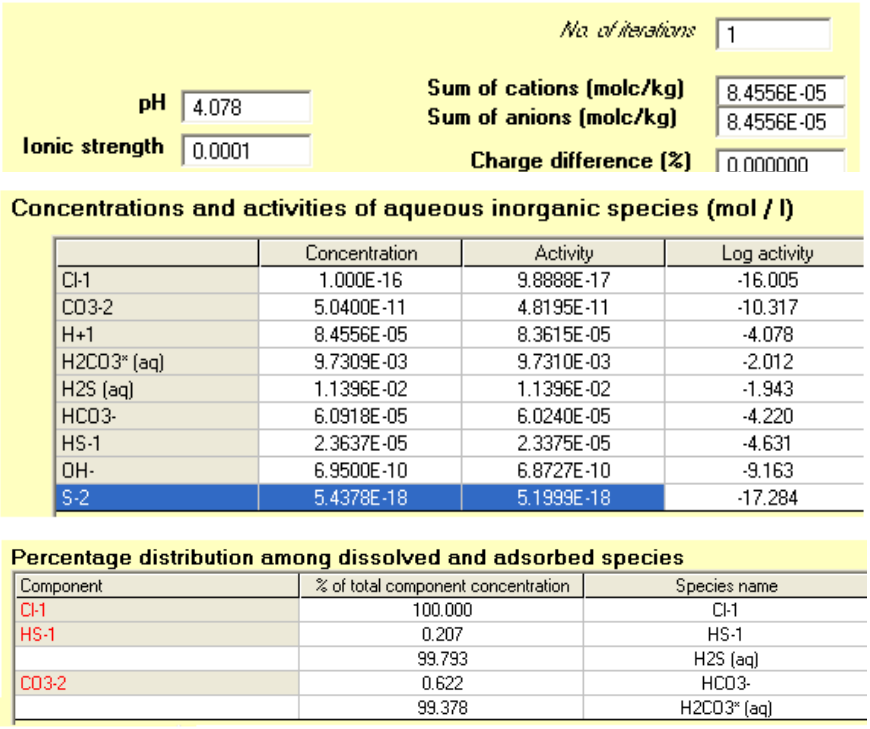
The lab scale reactor was run at 50 ˚C. Approximate solubilities at that temperature are put together in Table 4.6.

Table 4.6 Solubility of gaseous components in water (50°C)

Component	Molar wt.	Solubility	[mass]	Solubility	[molar]
H_2S	34	1.85 E+0	g gas/kg H_2O	55.07	mmol/l H_2O
O_2	32	2.7 E-2	g gas/kg H_2O	0.85	mmol/l H_2O
H_2	2	1.3 E-3	g gas/kg H_2O	0.66	mmol/l H_2O
CO_2	44	7.5 E-1	g gas/kg H_2O	17.25	mmol/l H_2O

4.4.2 Sour gases in the feed

Both CO_2 and H_2S influence the pH of the solution. Using Visual Minteq freeware software (Gustafsson, 2007), system equilibrium parameters were obtained. Figure 4.5 shows results obtained from the program for components in the gas feed (Table 4.3) for atmospheric pressure.



No. of iterations: 1

pH: 4.078

Ionic strength: 0.0001

Sum of cations (molc/kg): 8.4556E-05

Sum of anions (molc/kg): 8.4556E-05

Charge difference (%): 0.000000

Concentrations and activities of aqueous inorganic species (mol / l)

	Concentration	Activity	Log activity
Cl-1	1.000E-16	9.8888E-17	-16.005
CO3-2	5.0400E-11	4.8195E-11	-10.317
H+1	8.4556E-05	8.3615E-05	-4.078
H2CO3* (aq)	9.7309E-03	9.7310E-03	-2.012
H2S (aq)	1.1396E-02	1.1396E-02	-1.943
HCO3-	6.0918E-05	6.0240E-05	-4.220
HS-1	2.3637E-05	2.3375E-05	-4.631
OH-	6.9500E-10	6.8727E-10	-9.163
S-2	5.4378E-18	5.1999E-18	-17.284

Percentage distribution among dissolved and adsorbed species

Component	% of total component concentration	Species name
Cl-1	100.000	Cl-1
HS-1	0.207	HS-1
	99.793	H2S (aq)
CO3-2	0.622	HCO3-
	99.378	H2CO3* (aq)

Figure 4.5 System equilibrium and solutes concentrations

The above results can provide an explanation for the system running stably at pH 4, as this is the approximate equilibrium pH obtainable by feeding the system with site-specific geothermal gas. This could imply that the pH obtained in the lab scale experiment lowered due to sour gases dissolution in the broth rather than oxidation of sulfur to sulfuric acid and that the reactor was well-mixed and no H_2S limitation took place. Another conclusion (see Appendix A and Figure 4.4) is that H_2S solubility is a much stronger function of temperature than pressure, but the latter still may be the most significant factor in the provision of appropriate mass transfer rates.

4.4.3 Bioremediation need – H_2S

Given yearly mass flow of the H_2S and a preliminary goal on removal efficiency of ca. 95%, which sets a condition on minimum removal capacity of 850 kg of hydrogen sulfide per hour, approximately 600 m^3/hr of gaseous H_2S at standard conditions should be treated.

4.5 Bioreactor

The reactor decides the outcome of a process for which it was used, i.e. the success or failure of a project. Moreover, in most cases it cannot be easily replaced or redesigned without major changes to the rest of the installation. For those reasons the choice of a reactor type and configuration is one of the most decisive steps in the whole bioprocess design and should be treated with the utmost care. The following analysis will investigate potential choices and uncertainties regarding this part of the pilot plant.

4.5.1 Reactor sizing

Lab scale reactors usually do not exceed a volume of a couple of liters. Industrial plants make use of bioreactors as big as 250m^3. Pilot scale installations place somewhere between – usually in the range of couple of cubic meters. The size of the reactor itself not only dictates the requirements for all the flows in the process, but can also contribute greatly to the overall cost of construction and operation.

For the project, data from (Srivastava, et al., 1999) were taken to estimate the volume necessary to treat the amount of H_2S supplied according to the formula:

$$V_{reactor} = \frac{(\mathrm{H_2S\ supply}) \cdot (\mathrm{removal\ efficiency})}{(H_2S\ \mathrm{uptake\ rate}) \cdot (\mathrm{biomass\ concentration})} = \frac{\dot{m}_{H2S} \cdot \eta_{H2S}}{r_{H2S} \cdot X} \approx 50\,\mathrm{m}^3,$$

where: hydrogen sulfide uptake rate, r_{H2S} = 0.34 [g $(g_{BM})^{-1}(hr)^{-1}$ $(l)^{-1}$]; biomass concentration, X = 50g/l; mass flow of hydrogen sulfide from the power plant, m_{H2S} = 856 kg/hr; assumed necessary H_2S removal, η_{H2S} = 95%.

Discussion

Even though the volume does not seem to be very big, there are some pieces of data which burden the result with a high uncertainty. First of all, biomass concentration is taken as 50g $(l)^{-1}$ which, for a submerged fermentation, is very high. Typical obtainable densities are mostly in the range of 20g $(l)^{-1}$, unless immobilized cultures are used. High heat generation value would also imply that both the biomass concentration and specific productivity are set too high. Secondly, the hydrogen sulfide uptake rate is taken as 10 mmol (g biomass)$^{-1}$ (hr liter)$^{-1}$, which is one of the highest values reported (Syed, et al. 2006), (Lee, et al. 2006), (Beffa, et al., 1996). The mass transfer coefficient is taken from (Villadsen, n.d.) as the highest reported as well.

Finally, the reactor volume assessed in this manner gives the smallest necessary "active" volume, at highest possible parameters necessary to fulfill the objective of treatment. However, it is not possible to maintain, on a non-lab scale, uniform conditions over the whole bioreactor. Thus, the number obtained can be expected to be heavily underestimated – in the worst case, by an order of magnitude.

4.5.2 Reactor type

CSTR are well known for their issues with high energy demand when scaled up (Nielsen, Villadsen and Liden, 2003) – not reaching high mixing characteristics because of that. If deposition and abrasion are an issue, the reduction in the number of mechanical parts is beneficial. For that reason CSTR has been excluded in the primary study of a reactor type choice.

Biofilters and all other types of reactors with immobilized cultures are very efficient in bioremediation (Gabriel and Deshusses, 2003). There were examples of the successful, commercial operation of plants run for hydrogen sulfide removal in (Janssen, et al., 2000), (Janssen and Buisman, 1996). Nonetheless, the bioreactor type was also excluded from the considerations as well, as:

- it does not allow efficient biomass harvest and separation;
- gas-liquid mass transfer of hydrogen sulfide is based on scrubber-like operation with base in the solving liquid – the processes operate efficiently at pH above neutral, which is not the case of the project.

Narrowed choice forces leaning in the direction of airlift bioreactors, which are already proven in both gaseous fermentations and SCP production (Larsen, 2000) and provide numerous advantages at the same time:

- few moving parts, relative simplicity of construction;
- low energy use;
- low capital costs;
- high mass transfer rates[9].

Some of the obtained results allow further evaluation of the different types of airlift bioreactors depicted beforehand (see Chapter 2.5.4):

- reactors with separate downcomer and riser may substantially increase the reactor volume if the bubble residence time and flow will not be managed meticulously;
- jet loop design can save up on compression energy and increase bubble residence time;
- net draft tube type mass exchange between the downcomer and riser part is of great benefit in reduction of reactor size;
- U-loop reactors seem to be very promising, however to avoid reactor volume increase, the flow should be managed in a way that the bubbles are entrained with the flow, which contradicts the avoidance of bubble coalescence above certain superficial velocities;
- U-loop design facilitates the use of heightened pressure flows.

Preferred reactor type

Probably the most promising reactor type for the project is the deep-shaft airlift reactor depicted in Figure 4.6 taken from (Chisti, Mass transfer, 1999).

[9] In (Chisti, 1989), one of the only books on the subject, maximum achievable k_La for airlift bioreactors and oxygen gas was given as 0.1/s. In (Villadsen, n.d.), however, the value was said to be 1000/hr - around 3 times higher, which can imply rapid development in the construction and design or inconsistency of data. However, the value of 1000/hr was taken from a successful project running on methane, which is poorly soluble in water. For that reason the latter value will be assumed as the maximum obtainable.

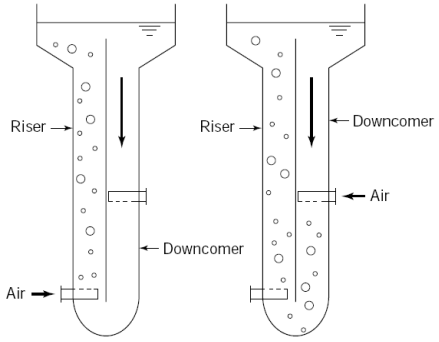


Figure 4.6 Deep-shaft airlift reactor

The main use of this type of design is in wastewater treatment. The operation starts with supplying gas into the riser to force liquid circulation. Then the gas is fed in the downcomer, instead of the riser, and the bubbles are entrained in the flow and forced down. The rise in the pressure, due to head increase over the bubble path in the reactor, increases mass transfer rate and keeps the gas in the liquid longer than the typical designs. Superior oxygen transfer rates and transfer efficiencies (on an energy basis) have been reported in (Chisti, 1999). The position also gives a much more detailed description of the reactor, its characteristics and operation.

4.6 Stoichiometry – substrate to product[10]

As the base case for the project, HOX bacteria for SCP production were investigated. The reaction for aerobic growth on CO_2 and hydrogen was determined to be (Ævarsson, 2008):

$$21H_2 + 6.5O_2 + 4O_2 + 1NH_3 \rightarrow 19H_2O + \text{Biomass } (4CH_{1.75}O_{0.5}N_{0.25}) \quad \text{(Eq. 4.1)}$$

Yet, as hydrogen sulfide was determined to be the preferred substrate, probably at reactor conditions, the HOX pathway is not fully utilized, if at all. For that reason, further calculations neglect hydrogen as an energy source for the reaction. Also, oxidation of H_2S is assumed to end at elemental sulfur (which can be referred to as the "first-step"); the reason for that being the pH control, which is currently made through the addition of a base. If oxidation would go further (to sulfuric acid), the base use would dramatically rise, increasing the cost, which is to be avoided.

Table 4.7 Bacterial reduced sulfur compounds oxidation

Reaction	**ΔG^0 [kJ/reaction]**
$H_2S + ½\ O_2 \rightarrow S^0 + H_2O$	-209.4
$S^0 + 3/2\ O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$	-587.1
$H_2S + 2\ O_2 \rightarrow SO_4^{2-} + 2H^+$	-798.2
$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2SO_4^{2-} + 2H^+$	-818.3

[10] In this chapter 1 mol of biomass corresponds to either 1 C-mol product or 1 mol of $CH_{1.75}O_{0.5}N_{0.25}$

A simple recalculation of the stoichiometric coefficients in accordance to their relative enthalpy of reaction[11] gives an approximated, integer-numbered reaction of biomass creation[12]:

$$24H_2S + 8O_2 + 4CO_2 + 1NH_3 \rightarrow 22H_2O + 4CH_{1.75}O_{0.5}N_{0.25} + 24S^0$$
(Eq. 4.2)

For that pathway, one can obtain mole-per-mole yields of product (biomass) versus each of the substrates:

Table 4.8 Mol/mol yield coefficients for substrates from Equation 4.2

Component	***Yield coefficient, $Y_{S/BM}$***	***Unit***
H_2S	6	mol/mol biomass
O_2	2	mol/mol biomass
CO_2	1	mol/mol biomass
NH_3	0.25	mol/mol biomass

Now, having a bit different than typical (from the literature) biomass composition, after recalculation of its molar mass, the table of substrate to product demand (yield coefficients on both molar and weight basis) is given:

Table 4.9 Yield coefficients for substrates according to Equation 4.2

Component	***Per kg biomass***		***Yield (molar basis)***	***Yield (wt. basis)***
			mol /mol biomass	kg / kg biomass
H_2S	237.6	mol	6	8.08
O_2	79.2	mol	2	2.54
CO_2	39.6	mol	1	1.73
NH_3	9.9	mol	0.25	0.17

If ca. 8kg of hydrogen sulfide are necessary for the creation of 1kg of SCP, at 2500t per year, the amount of H_2S available on-site will not be sufficient (see Table 4.1); which would mean that, according to Eq.4.2, only 37% of projected yearly SCP production could be achievable using only first step oxidation (to elemental sulfur).

[11] The enthalpies were not corrected for temperature, as the data and calculations themselves are burdened with probably around 25% uncertainty.

[12] See the end of the subchapter 4.6 with discussion of the results

4.6.1 Maximum H_2S uptake rate vs. energy metabolism

To obtain a certain amount of product (in this case biomass) an appropriate growth rate has to be maintained. On the other hand, the H_2S uptake rate is already limited at a certain level (r_{H2S}). The given biomass production rate cannot be maintained by oxidation to elemental sulfur only (according to the previous paragraph). Thus, the actual pathway of the reaction has to be different. Once again referring to enthalpies of reactions and excluding hydrogen from the divagations, a set of balancing equations for oxidation to S^0 and sulfuric acid can be obtained:

$$\frac{H_2S\_uptake\_rate}{\mu \cdot M_{H2S}} = x + y$$

(Eq. 4.3)

$$\Delta H_S x + \Delta H_{H_2SO_4} y = \Delta H_{H_2} \cdot \frac{21 \cdot 1000}{4M_{BM}}[mol]$$

(Eq. 4.4)

Equation 4.4 compares energy from the oxidation of hydrogen (Eq. 4.1) to the oxidation of hydrogen sulfide to S^0 and sulfuric acid for the creation of 1kg of biomass[13], whereas Equation 4.3 relates the hydrogen sulfide oxidation rate to the hydrogen sulfide uptake rate (r_{H2S})[14].

Where:

x – moles of H_2S oxidized to elemental sulfur per 1kg biomass

y – moles of H_2S fully oxidized (to SO_4^{2-}) per 1kg biomass

Table 4.10 Mixed complete and incomplete hydrogen sulfide oxidation

Oxidation to → / ***Property↓***	S^o	SO_4^{2-}
Mol oxidized per kg biomass	34,1	53,5
Relative energy gain from oxidation	14.3%	85.7%
By-product created/ kg BM	1091 g	5240 g
moles of H_2S oxidized/ mol biomass	3,44	5,40

[13] It is assumed that creation of biomass requires the same amount of energy, whatever metabolic pathway was used

[14] Amount of hydrogen sulfide for oxidation, equal to H_2S uptake rate has to suffice for creation of 1kg of product

4.6.2 Corrected metabolic pathway

Using the data from the previous subchapters, a reaction for biomass creation can be rewritten under certain assumptions[15]

$$8.84H_2S + 8.52O_2 + 4CO_2 + 1NH_3 \rightarrow 6.84H_2O + 4CH_{1.75}O_{0.5}N_{0.25} + 3.44S^0 + 5.4SO_4^{2-}$$

(Eq. 4.5)

Discussion of the results

The final reaction stoichiometry that was determined still cannot be taken as certain. First of all, the enthalpy ratio scaling does not have to reflect actual phenomena in the biological systems. For example, S^0 to SO_4^{2-} oxidation can be given. Even though the step gives around three times more energy than the hydrogen sulfide to sulfur oxidation, it is not the preferred one. The most probable reason for that may be less favorable kinetics or the fact that different oxidation steps are carried out by different strains in the culture. Their activity, on the other hand, depends on the conditions in the reactor.

What is more, it cannot be assumed that no hydrogen is used, as the culture is not isolated and the conditions are not sterile. Also, the C-mole P/S ratio is given as unity. For the typical processes involving aerobic strains, values in the range of 0.4-0.7 are reported, depending not only on the process, but also the conditions.

Furthermore, there are other effects involved – like the reported shift to complete H_2S oxidation under oxygen limitation (Takeuchi and Fujioka, 1995). Finally, the estimations themselves are burdened with probably no less than 10-20% uncertainty.

4.7 System components' toxicity and their influence on the operation

Bacteria, even though they are in general resistant, show only a limited capacity for the presence of some compounds in their environment. As was previously discussed, there can be substrate or product inhibition due to its concentration. Therefore, it may be necessary to investigate the threshold at which all the system components should be maintained to keep the culture running smoothly.

4.7.1 Hydrogen sulfide

For most organisms, hydrogen sulfide is lethal at sufficiently high concentrations. The level, however, varies between the species. Even if some technologies have high daily throughput in terms of sulfur compounds, it does not mean that they are being fed concentrated. When expressed in ppmv, the typical maximum level of H_2S that can be handled by the bacteria is stated to be 2000. Studies at the University of Akureyri in the batch cultures (Reynisóttir, 2007), (Vésteinsdóttir, 2008) confirm that this level is achievable. In the lab scale experiment, the gas was fed with 4000 ppmv (4% vol.) H_2S, which already by far exceeds what was reported in the literature. The highest biologically treated H_2S concentration found in the literature was 10% (v/v) (Stristava and Walia, 1999). In the final process, hydrogen sulfide will be given at concentrations that may reach

[15] No hydrogen is used, excessive hydrogen from oxidation of H_2S goes into water on reaction's right side, SO_4^{2-} is used to denote complete oxidation to sulfuric acid, although for stoichiometry to be correct, it should be SO_3

up to ca. 20% (v/v). Thus a question arises, whether the level will be lethal for the bacteria or not.

Up till now, there were no signs of H_2S being harmful for the culture, but at the final assumed level, it could be. It is not yet clear what concentration prevails over – H2S (g), H2S (l) or [HS^-]. It may be that the [HS^-] concentration is the one that can be toxic and at low pH the amount of this species in the liquid phase is lower by two orders of magnitude than at pH 7, which could explain bacterial resistance to this feed component.

Still, as a general rule (Doran, 1995), the maximum growth rate and the highest productivity (hydrogen sulfide uptake rate) should not be expected at the same concentration, which can be illustrated in Figure 4.7. However, the exact relation is yet to be found.

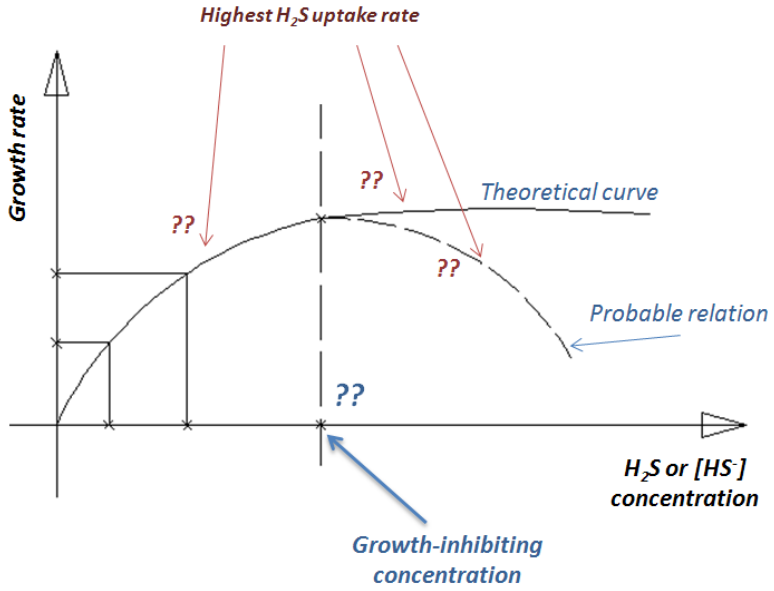


Figure 4.7 Growth and uptake rate vs. H_2S concentration (Kristjánsson, 2008)

4.7.2 Nitrogen source

It was not yet determined in which form the nitrogen source would be introduced into the system. It was reported (Nielsen, Villadsen and Liden, 2003) that if, under culture conditions, nitrogen is present in the form of NO^{2-} ions in the broth, it can cause substrate-level limitation even at relatively low concentrations. That fact can be especially important, as one of the aims of the process is to grow as much biomass as possible and nitrogen, accounting for over 10% of bacterial mass, has to be introduced into the reactor in significant amounts.

4.7.3 CO_2 and O_2 toxicity

Even though in general neither carbon dioxide nor oxygen (if in amounts appropriate for bacterial growth) affect the cultivation, there were some reports of the toxicity of both compounds at high partial pressures. In the case of O_2 there seems to be a low level of risk, as the issue lies in its provision at sufficient quantities rather than excess; however one should note that in the typical environment for sulfur-oxidizers, oxygen is rather scarce. It also does not seem to be very probable that a high concentration of CO_2 could be limiting,

due to low pH tolerance of the culture and its CO_2-fixing character. Yet at some point, the broth will be CO_2-saturated and the chance of substrate-level limitation can become an issue.

4.8 Substrate limitation

For full scale plants it is not possible to provide uniform conditions and perfect mixing over the whole volume of the reactor. Also, certain needs of the culture can be contradictory, thus leading to certain limitations – not because of external reasons, but because of the nature of the bioprocess itself.

4.8.1 Mass transfer-induced – Oxygen demand calculation

The most common problem in scale-up is connected with a decrease in mass transfer intensity. This is especially important for gas-fed fermentations. For aerobic cultures usually the biggest problems lies in sufficient oxygen supply. An example of the estimation of necessary parameters for the project is given below.

To begin with, one can calculate oxygen demand, based on wt., from the reaction derived beforehand (see Chapter 4.6) as:

$$Y_{P/O}[\frac{kgO_2}{kg_{BM}}] = \frac{1000M_{O2}}{M_{BM}} \cdot (Y_{P/O}[\frac{molO_2}{mol\_BM}]) = 2.70\frac{[kgO_2]}{[kg_{BM}]}$$

On the other hand, the culture's oxygen demand has to be met by sufficient supply through mass transfer operation, which leads to the formula:

$$\frac{Y_{P/O}}{M_{O_2}}\mu[\frac{kg}{kgBM \cdot m^3 \cdot hr}] \cdot X[\frac{kg}{m^3}] = k_L a[\frac{1}{hr}](c^*_{O_2}\ [mmol] - c_{O_2}\ [mmol])$$

Which gives conditions for concentration differences between the phases:

$$(c^*_{O_2} - c_{O_2}) = 482\frac{1000}{k_L a[\frac{1}{hr}]}[\mu mol]$$

With data from the literature (Villadsen, n.d.): c_{O_2} at 20 µmoles and $k_L a$ = 1000, oxygen mass transfer will not be limiting if:

$$c^*_{O_2} > 500\ \mu mol$$

The Table 4.11 puts together concentration values of oxygen fed at various parameters and the percentage of reactor under oxygen limiting conditions.

Table 4.11 Oxygen feed concentration as a limitation factor

Oxygen feed type	$c^*_{O_2}$ [µmol][16]	Percentage of reactor with first order kinetics
Air, 21% oxygen, 1 bar	180	100%
Pure oxygen, 1 bar	855	59%
Pure oxygen, 2 bar	1700	29%
Pure oxygen, 3 bar	2550	20%
Pure oxygen, 4 bar	3400	15%
Pure oxygen, 5 bar	4250	12%

One should remember that full utilization of oxygen is undesirable, as it would lead to unacceptably high reactor volumes (Villadsen, n.d.).

4.8.2 Hydrogen sulfide mass transfer

If the case with oxygen mass transfer can be quite easily simplified and has been thoroughly researched, for the hydrogen sulfide and the gas feed mixture the issue becomes much more complex.

Hydrogen sulfide solubility in water is much greater than that of oxygen. As for more soluble compounds, liquid side interface resistance starts to play a bigger role and should not be neglected without previous estimation.

The pH at which the reactor operates greatly influences the H_2S dissociation and species distribution, which at higher pH can be compared to mass transfer intensification by facilitated diffusion. Biological processes – basically substrate uptake – result in further increase of transport coefficients over the values predicted by the theory. On the other hand, if the H_2S dissolution rate is said to be controlled mainly by partial pressure of the gas, at high CO_2 concentrations in the system it was reported to reduce greatly (Kohl and Nielsen, 1997). That could only prove that multi-component mixtures cannot be treated carelessly by relatively simple equations, correct for single-component cases. Furthermore, the presence of solids in the broth hinders mass transfer as well, leading to even bigger uncertainty in the actual H_2S mass transfer coefficient estimation.

All in all, at bioreactor conditions, hydrogen sulfide's solubility is still larger than that of oxygen by two orders of magnitude. Nonetheless, taking into account relative substrate-product demand and species distribution, it does not seem possible to encounter severe substrate-level limitation, because of mass transfer only[17], if gas residence times are kept at reasonable levels. Thus, if oxygen transfer can be handled properly, hydrogen sulfide feeding should pose no more problem than the latter.

[16] Assuming that condition c ~ p is met for such pressures; values are rounded down to nearest 50-ty for pressures higher than 1 bar

[17] Were that to happen, sulfur or hydrogen oxidation would dominate – possibly slowing down the growth, yet still keeping the culture running.

4.8.3 Substrate availability

The study shows that for the particular application, resource availability will not be an issue, as hydrogen sulfide amount dictates the removal capacity and bioprotein production. In a more general case, however, site availability of a substrate should be investigated.

If H_2S was not used as an energy source, hydrogen gas would have to be the sole electron donor. A report (Prokaria ehf., VGK, 2005) shows that to completely deplete the carbon source from the geothermal gases, the amount of hydrogen in it is not sufficient. The additional demand would have to be met by external provision of H_2 – having significant impact on the economic balance – and one should keep in mind that the presence of hydrogen in NCG, at such high concentrations, as in Iceland, is not common elsewhere.

Conclusions

All of the abovementioned confirm the general ambiguity of the bioprocesses. The growth and metabolic activity, even if coupled, call for a hard compromise between the seemingly divergent objectives – in this case, the bioremediation of geothermal gases and production of bioprotein. The aim of efficient gas treatment will not be achieved without either an increase in reactor volume and O&M costs or a decrease in SCP production.

Another thing that can be derived from the chapter is that the one step process (oxidation to sulfur only) is probably not feasible at all under the current assumptions[18]. Either mixed or simultaneous $H_2S \rightarrow S^0$ and $S^0 \rightarrow SO_4^{2-}$ steps seem to be more viable.

4.9 Cooling/heating load

Based on typical values (Doran, 1995), (Villadsen, n.d.) the heat of the reaction with oxygen can be estimated as:

$$E_{gen}[\frac{MJ}{kg\_SCP}] = \frac{0.46 Y_{so} \cdot 1000}{M_{BM}} = 39.2 \text{ [MJ / (kg biomass)]}$$

That gives an average heat load, from biomass creation, of 62 kW/m^3 and 3.1MW_{th}, in total. Other types of heat generation in the reactor due to energy dissipation can be neglected (Chisti, 1989), (Villadsen, n.d.). Still, the numbers obtained through calculations are larger than the ones typically reported by a factor of ten. It gives another argument supporting the doubt about the feasibility of running the plant on current assumptions. A mix of following is probably necessary for the cooling load to be appropriate: lower biomass concentration, bigger reactor, lower productivity.

Even though the geometry is not given yet, it is almost certain that external cooling will be needed. However, due to the presence of sulfur in the reactor, because of deposition and scaling issues, an internal heat exchanger should be avoided.

4.10 By-products

In most processes, apart from the product, there are large quantities of by-products which have to be utilized or disposed of. Treatment of those flows increases the costs and, in the case of low-value products, can significantly decrease the economic result of the whole

[18] Regarding reactor parameters and culture used

process. It is necessary to assess possible disposal pathways for all the unnecessary products or find a way to make the best use of them.

4.10.1 Elemental sulfur

Elemental sulfur is created during the first oxidation step. In the water, sulfur conglomerates and precipitates as a soft solid mass. If the bacteria are deprived of their primary energy source (H_2S) they tend to stick to the sulfur particles created beforehand and begin the oxidation of the latter.

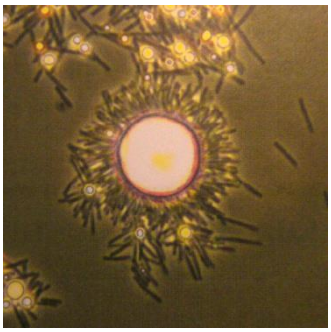


Figure 4.8 SOX Bacteria sticking to sulfur particles (Reynisóttir, 2007)

Elemental sulfur does not seem to have much impact when present in low quantities in animal feed (Ævarsson, 2008).

4.10.2 Sulfuric acid

If there is not enough energy to gain from the first oxidation step, H_2S goes all the way to SO_4^{2-}, which, in the presence of water, produces sulfuric acid. It is one of the strongest inorganic acids, fully dissociating in solvent. High pH reduction of the fermentation broth and effluent streams can be expected if the metabolic pathway ends with complete H_2S oxidation.

4.10.3 Disposal

Table 4.12 Possible by-product disposal options

By-product	Disposal way	Issues
S^0	Storage	Already lots of solid material stored all over the world, intermediate disposal solution
S^0	Chemical substrate	Certain grade (purity) has to be achieved for chemical processes; to obtain high price, high purity (and processing) is necessary; there are two main types of sulfur in solid state which vary slightly in their properties – the type from the production plant is not identified

S^0	Fertilizer	Sulfur is also one of the elements necessary for plant growth; Icelandic soil is deprived of the compound, which is simply washed out. Some amounts of sulfur could be sold as a fertilizer for farmers, though it is believed that the amounts would be small in comparison with the predicted production from the plant
Sulfuric acid	Geothermal plant fluid enhancer	Scaling is one of the primary issues in the operation of geothermal plants; lowering pH was proved to abate some of the issues with silica precipitation (Takuechi, et al. 2000), (Kudo and Yano 2000); there were tests carried out with bioreactor providing the acid for the purpose (Takeuchi and Fujioka, 1995)
Sulfuric acid	Mixing with the geothermal reinjection fluid	As with CO_2 and H_2S reinjection the properties of the fluid, mainly the pH, vary from the ones obtainable typically; there is a high chance of water-rock interactions in the vicinity and the reinjection well itself; certain test are carried out to determine the safety of such a disposal option (Ævarsson, 2008)
Sulfuric acid	Chemical agent	Use of sulfuric acid in the chemical industry is widespread, yet with the quantities obtainable there is no proper sink for all the by-product in Iceland. The two most promising options are production of sulfates (fertilizer) and gypsum

4.11 Downstream processing

The product obtained from the reactor is by no means ready for sale or consumption. There have to be certain processing steps involved to ensure safety, proper physical properties and permit storage. As was previously stated, for low-value products, downstream processing cost can be the biggest obstacle in the bioprocess development. Case study-relevant steps and requirements will be discussed in the chapter.

4.11.1 Case analysis

Products obtained from fermentation need to be recovered from a broth, when a submerged type process is deployed. In this case, methods for liquid-solid separation have to be used. In the solid phase sulfur is also present. The risk of clogging and deposition makes one discard ultrafiltration – a common practice – right away. Another problem which is caused by sulfur is the adhesion of bacteria to the sulfur particles. It has been estimated that in the broth from the experiment around 50% of the total biomass is suspended in the liquid and the rest attaches itself to the solid particles.

The biomass – mainly protein – has very high nucleic acid and nitrogen content, which makes it unfit for direct consumption. Biomass contamination has to be prevented as well. Also, mainly to avoid organic matter degradation over the storage period, water content has to be greatly reduced – starting from a couple percent of solids in the broth all the way to 10-20% moisture content in the final product, on a wet basis.

4.11.2 Biomass recovery

Was the process run with complete hydrogen sulfide oxidation only, the final effluent stream to process would be much easier to handle. However the first step oxidation, chosen as the primary process at the moment, induces the separation of biomass and sulfur, which are both present in the solid phase. One way of doing that would be sedimentation, though the biomass recovery would be unacceptably low. Thus, the washing step will probably be a very promising solution (Kristjánsson, 2008); however the efficiency of the process was not estimated. There is also a chance that the streams obtained before and after the washing could require separate processing and conditioning paths (Kristjánsson, 2008). More sophisticated methods of making the bacteria separate from sulfur could involve treatment in a high ionic strength environment, which would disturbe the cell potential (Ævarsson, 2008), though that has not been investigated yet.

4.11.3 Thickening

It can be shown that agglomeration of sulfur causes a significant difference in biomass and bacteria size. Apart from that, if the assumptions for biomass concentration will be met, 5-10% of solids can be anticipated in the liquid harvested from the reactor. Both factors advocate the use of a settling chamber to allow particle sedimentation and thickening of the broth, which could greatly reduce the downstream processing costs.

Centrifugation is another common step in the reduction of water content. As the bacteria did not show susceptibility to shear stress, there are no contraindications to include this step in downstream processing.

Final water content reduction occurs during spray drying.

4.11.4 Biomass conditioning

Bacteria can have even 15% wt. of genetic material in the cells, primarily RNA. To ensure safety, it has to be certain that it was completely inactivated. Foreign and viral contamination has to be prevented as well. A common practice involves shock heat treatment and the use of UV radiation.

SCP for consumption and feed has to meet certain standards, composition being just one of them. With a high rate of certainty, it would be either very costly or impossible at all to remove all the sulfur present in the final product. Apart from separation being imperfect, internal sulfur deposit creation in bacteria cannot be excluded and can reach 10-15% by weight (Don, et al. 1994). Still, if the product were to be just a component mixed into e.g. fish feed the sulfur content (low and probably harmless at such concentrations) would be more than offset by the protein content.

4.11.5 Product safety

Each bioproduct has to undergo some investigation for safety of its use. In the seventies The Protein Advisory group of the United Nations issued guidelines regarding novel protein sources, including SCP (Litchfield, 1978). However for single-cell protein, there is still very little said about any specific regulations with which it has to comply, even on the FAO website. It is certain that the nucleic acid content has to comply with FAO regulations; however it seems that apart from that, the final product has to follow the codes of respective countries. For the United States, the main responsible authority is the Food and Drug Administration.

Apart from the product itself, the plant also has to fulfill regional standards of any kind. The discussion about typical issues, also regarding safety – very relevant because of H_2S handling in the project – can be found in (Marvin and Wilson, 1999).

5 PROJECT'S FUTURE PROSPECTS

Although the project has very ambitious objectives, it will probably not be possible to develop it under the current assumptions. Possible future project development is discussed further in this chapter.

5.1.1 One- vs. two-step process

The hydrogen sulfide in the feed gas can be oxidized to elemental sulfur or sulfuric acid as discussed in Chapter 4.6. The two processes could possibly be separated in time, leading to a two step approach, meaning H_2S to S^0 oxidation and S^0 to sulfuric acid oxidation, respectively. A schematic representation is shown in Figure 5.1.

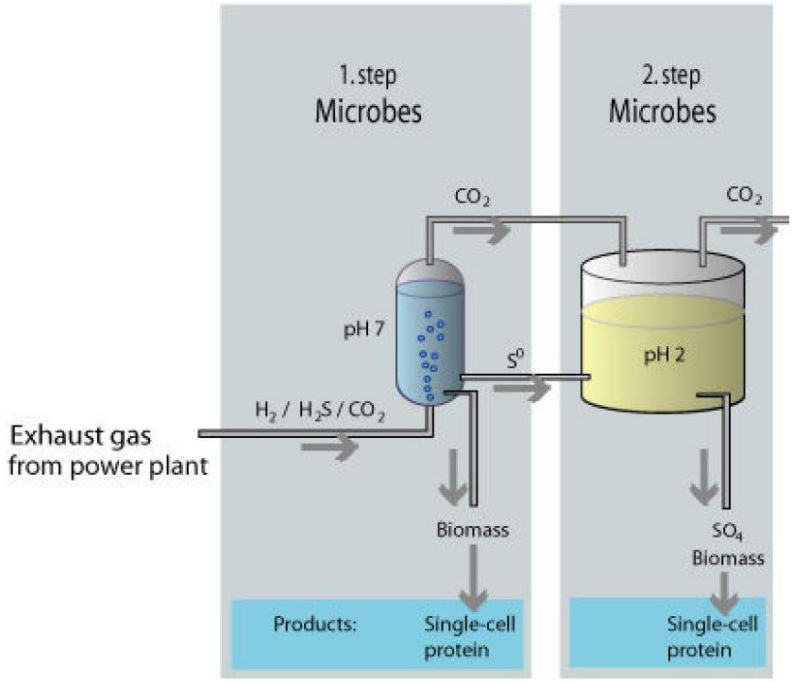


Figure 5.1 Two-stage approach to hydrogen sulfide oxidation in the GEOGAS project

Assessment of parameters made in Chapter

4.6 shows that at current assumptions regarding the hydrogen sulfide uptake rate, it is impossible to run single stage oxidation to sulfur only. In other terms, such a high biomass concentration at a fixed uptake rate would lead to a 2/3 reduction in SCP production, if only the first oxidation step was present. The other way to have only the first-step process would be to have the concentration of biomass, at a fixed growth characteristic, no greater than ca. 18 g/l. The number seems to be much closer to the typical submerged fermentations' parameters than the previously projected 50g/l. Another reason for the biomass concentration being estimated too high is the calculated heat generation due to biomass creation (see Chapter 4.9) – around ten times what is usually reported in literature (Nielsen, Villadsen and Liden, 2003).

The fact of sulfur to sulfuric acid oxidation has a number of serious consequences for possible project outcome. First of all, at good reactor mass transfer characteristic,

pH 4 can be expected from just the dissolution of the feed gas into the fermentation media. Then, if the bacteria were to go into second-step oxidation, the pH would go down dramatically, making the operation cost soar because of pH control by the use of a base.

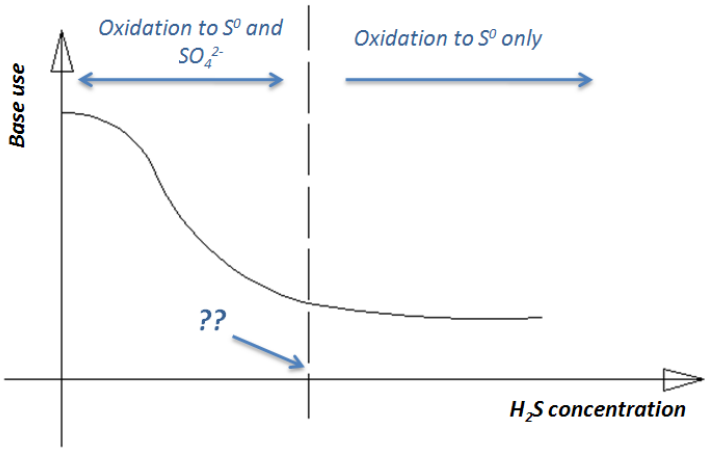


Figure 5.2 Base use increase under H_2S limitation (Ævarsson, 2008)

This is obviously unacceptable, as the key advantage of the project is the use of a virtually free fermentation substrate, but relatively expensive mineral medium and nitrogen source in significant amounts, dictated by the SCP composition (see Table 4.5).

Then it should not be expected that single-pass of the feed gas will be enough to achieve H_2S removal efficiency of ca. 95%. It is true that removal efficiencies of 99% or higher were reported (Syed, et al., 2006), yet most of them were for biofilter constructions and at much lower hydrogen sulfide concentrations. The fact that up to 40% of the gas retention in the appropriately designed gas separator of ALR is possible (Merchuk and Gluz, 1999), still it will not cover that up. Reasons for that were discussed in Chapter 4.5.1 and are also illustrated in Appendix A (note that oxygen's dimensionless Henry's coefficient, at the given temperature is equal to around 30 – roughly 80 times more than the one for hydrogen sulfide).

All of the above imply that, rather than aiming at a single-stage process, the separation of the two oxidation steps should be pursued. In that case, the first step could be done with an immobilised culture based on biofilter/bioscrubber design (see Chapter 2.5.7) and the other one with an ALR. The solution however, if doable, would be more costly. There would be a need for not one, but at least two reactors. The streams obtained from both of them would have different characteristics and, requiring separate handling (Kristjánsson, 2008), complicate further downstream processing – one of the biggest cost-contributors in low-value substrate fermentations.

5.1.2 Geothermal power, bioprocess combine

Even if the project will not be as profitable as expected, due to lower SCP output there are still some windows for opportunity to make it more attractive. Several studies (Takuechi, et al., 2000), (Kudo and Yano, 2000) and patent applications (Takeuchi and Fujioka, 1995) showed the possibility of silica scaling abatement through biological means. The problem occurs most of all during the utilization of high temperature areas (DiPippo, 2007) present not only in Iceland, but also in, e.g., Japan, Philippines and New Zealand. Lowering the pH of the power plant's working liquid could provide additional benefit in the form of increased power output thanks to lowering the temperature of the liquid for reinjection.

Then again, the geothermal power plants may be forced to introduce means for hydrogen sulfide removal and CO_2 emission reduction. The amount that would have to be spent on the cheapest clean-up technology, redirected into bioprocess operation, could make the economic balance of it positive.

5.1.3 Three step process

For the two step process, the carbon source would come from the CO_2 in the feed gas. The amount of carbon dioxide used for this approach would not exceed 3000t/yr; which, at quantities present at the site, results in no more than 15% CO_2 abatement. Therefore, the most complex but also most promising shape of the project development was proposed – a three-stage approach. A diagram showing the general concept is presented in Figure 5.3.

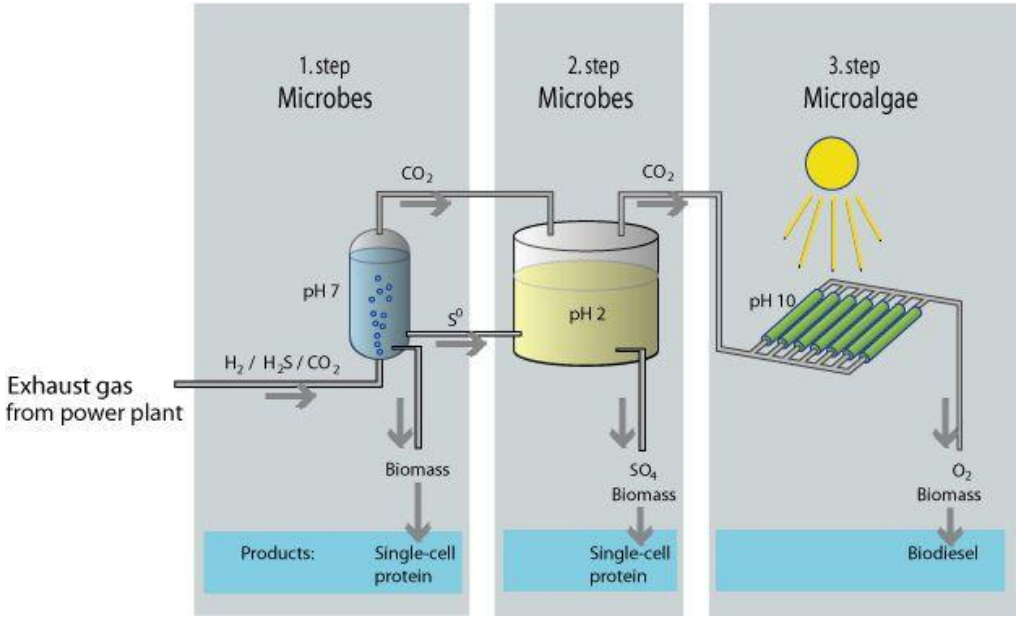


Figure 5.3 Three-step approach in the GEOGAS project

Rough estimates show that full utilization of CO_2, available only at the Nesjavellir site, could fuel up to 1% of Iceland's diesel-driven automobile fleet, not to mention doubling the revenue from the bioproducts sales.

5.1.4 Biorefinery concept

A biorefinery is a facility which produces fuels, heat and power, as well as chemicals from biomass (Clark and Deswarte, 2008). The key behind the potential biorefinery concept's success lies in its innate ability to fully utilize biomass' constituents through the production of a wide range of products, thus maximizing the value of the final products. One example of such a portfolio was proposed by (NREL, n.d.):

- High-value bio-chemicals in low quantities for enhanced profitability;
- Large volumes of liquid bio-derived transportation fuel for CO_2 abatement and increased energy security;
- Heat and power for own needs, possibly sale into the national grid, reducing GHG emissions at the same time.

In such a case, the three-step approach to the GEOGAS project could fulfill all the requirements for such a facility. The algae would produce biodiesel and possibly provide biomass for further fermentation, and the cooling demand from the two first steps could be used to cover heat demand for algae cultivation or drive a low-efficiency Stirling engine. The main low-value product would still be SCP for feed or food and the profitability could be enhanced through production of expensive bio-chemicals such as enzymes or specialty chemicals, which were investigated during the preliminary project preparation phase (Ævarsson, 2008). All of them would probably require genetic or metabolic engineering, however nowadays process-oriented strain (re)construction seems to achievable.

5.2 Concluding remarks

Pollution is nothing but the resources we are not harvesting. We allow them to disperse because we've been ignorant of their value.

Buckminster Fuller

Twenty years ago hardly anyone had heard about such a thing as *industrial ecology*. Its main idea is the imitation of nature, where what is a waste in one process is used as input to others. In 1997, the Journal of Industrial Ecology was established. There is still a debate on CCS technologies as a remedy to a carbon and oil constrained world. Another proposed option for reductions in carbon dioxide emissions from transportation was algal biodiesel. A report by (NREL, 1998) stated that such a method for alternative fuel production is too expensive and there were too many obstacles for it to be feasible in the near future. In the article by (Chisti, 2008) it is said that the costs of production of algal biomass would have to decline by a factor of 9 to be competitive with petroleum-based fuels with an oil barrel price around $100. In April 2008, the first commercial biodiesel algae-based plant started operation (Cornell, 2008). Now, February 2009, the barrel costs around $40 and the company, which made the first plant, still is up and running.

What does that prove? Basically, if something is not viable now, it probably will be soon. Waste streams should no longer be regarded as a nuisance, but rather a huge potential to tap, both environmentally and financially.

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Appendix A – Hydrogen sulfide solubility data

Solubility of H_2S in water expressed as Henry's law constant

Pressure [atm]	H [10^2 atm/mole fraction]						
	5	10	20	30	40	50	60
1	3.12	3.64	4.78	6.04	7.35	8.65	9.81
2	3.19	3.69	4.80	6.06	7.39	8.77	10.02
3	3.26	3.72	4.83	6.09	7.42	8.83	10.11

Adapted from (Kohl and Nielsen, 1997)

Equation: p = Hx, where: p – partial pressure of solute in the gas phase [atm]

x – mole fraction of solute in the liquid phase

One can recalculate values given below into the dimensionless Henry's constant using factor 6.389E-01 (Sander, 2007).

Vapor-liquid equilibrium system H_2S – CO_2 – CH_4 – H_2O, for temperatures between 85-115 °F and pressures from atmospheric to 1,014 psia, as reported in (Kohl and Nielsen, 1997)

$$K_{methane} = 306{,}000/p + 2.19t + 3.910t/p - 145.0AG - 121.6R$$

$$K_{CO_2} = -3{,}500/p + 0.12t + 360t/p + 8.30AH - 5{,}825R/p$$

$$K_{H_2S} = 4.53 - 1.087/p + 110.0t/p + 4.65AG$$

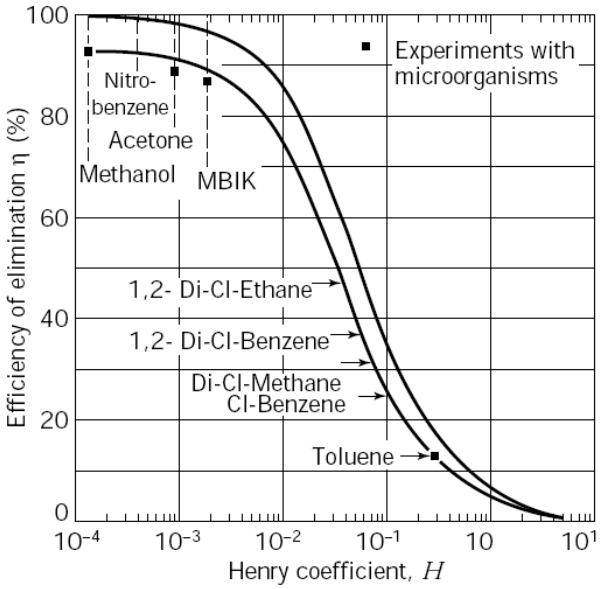
Where: K – mole fraction in gas phase/mole fraction in water phase;

p – system pressure, psia;

t – system temperature, °F;

AG – mole fraction CO_2 + H_2S in the gas phase;

R – mole fraction, H_2S/AG.



Elimination efficiency as a function of Henry's coefficient in a single-plate bioscrubber, taken from (Friederich and Werner, 1999)

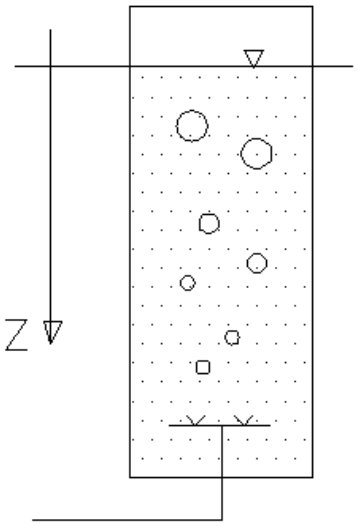
Appendix B – Model for estimation of gas holdup

Gas holdup is a difficult parameter to estimate, even when the geometry is known (Chisti, 1989) – which, in the case of the project, is still to be decided. For that reason an oversimplified model of gas behavior in the riser part was made.

Following are assumed:

- The whole reactor is a bubble column;
- The gas obeys the ideal gas law;
- The headspace has pressure of 1 bar, equal to ambient pressure;
- There is no mass transfer, heat exchange and slip between the phases;
- Liquid is stagnant;
- Pressure is dependent only on the water head.

Description of the model is given below:



H = z(max) = z(sparger)

$$p(z) = 1 + p(H)\frac{H - z}{H}$$

$$p\dot{V} = \dot{m}R^{*}T = C$$

$$\dot{V}(z) = \frac{C}{p(z)}$$

$$\overline{\dot{V}(z)} = \frac{C}{H}\int_{0}^{H}\frac{dz}{p(z)} = 10\frac{C}{z}\ln(1 + \frac{H}{10})$$

p(z) in [bar], z in [m], V in [m^3/s], p(z) is only a function of water head,

pressure in the head space = 1 bar, gas holdup $\varepsilon = \frac{V_G}{V_G + V_L} = 0.2$, gas holdup time in [s],

NCG flow is taken as 0.754 m^3/s, reactor volume = 50 m^3

H = Z(max) [m]	Mean volumetric flow [m^3/s]	Average GH time [s]
0	0.754	13.3
1	0.719	13.9
2	0.687	14.6
5	0.611	16.4
10	0.523	19.1
15	0.461	21.7
20	0.414	24.1
25	0.378	26.5
30	0.348	28.7
40	0.303	33.0

Now, the model can be slightly modified. The assumption about headspace pressure is changed in such a way that the pressure there is a result of the substraction of maximum head from the feed pressure, as given below.

Overpressure (absolute) in the head tank $\Delta p = p(gas\_feed) - 0.1H$ [bar]

The resulting, modified equation for volumetric gas flow, over the whole reactor is as follows:

$$\overline{\dot{V}(z)} = \frac{C}{H}\int_0^H \frac{dz}{p(z)} = 10\frac{C}{H}[\ln(\Delta p + \frac{H}{10}) - \ln(\Delta p)]$$

Table of gas holdup times,[s] = f(H, p_{feed}) is given below.

H[m]	*Feed pressure [bar]*				
↓	**1**	**2**	**3**	**4**	**5**
5	--	23.1	36.4	49.7	62.9
10	--	19.1	32.7	46.1	59.4
15	--	--	28.7	42.3	55.8
20	--	--	24.1	38.3	51.9
25	--	--	--	33.8	47.8
30	--	--	--	28.7	43.4

Conclusions:

- gas flow to be fed into the bioreactor has to be subjected to contraction due to pressure, otherwise it will be too big compared to gas holdup, which airlifts can handle;
- Parameters in most cases are very similar to the Norferm project, so similar requirements should probably be met on the most important flow-dependent factors. Yet, the gas residence time, if the design is to be the U-loop type, will probably be much smaller;

  // 60 [s] GH time coupled with ca. 30 [m] of downcomer-riser at volume ~ 10 [m^3] gives mean gas velocity (in the reactor) in the range of 0.3-0.7 [m/s], which, without a neat engineering solution – not identified as of now – is not possible //
- As shown in the tables above, overpressure in the headspace of the reactor can allow bigger mass gas flows at the same reactor-averaged volumetric coefficients, enhancing mass transfer as well;
- GH time is most likely underestimated – mass transfer should increase it, solid content in the liquid will decrease it, turbulent flow will increase it; in the end, with a high degree of certainty, actual GH time will be in the range of 1-2.5x what is given in the tables (for type I model); the model with the overpressure in the top space can be expected to be less faulty, thus the result should not be that overestimated;
- The most preferable gas bubble size (to be fed into the reactor) is between 3-6 [mm], bubbles bigger, as well as smaller, than 1 [mm] in diameter should be avoided (Doran, 1995);

Appendix C – Examples of correlations for gas holdup

Empirical expressions for gas hold-up in external-loop ALRs taken from (Merchuk, 1999)

No.	Formula
1	$\varphi_r = \frac{0.6\rho_G^{0.062}\rho_1^{0.069}\mu_G^{0.107}}{\mu_1^{0.053}s_1^{0.185}} \cdot \frac{J_{Gr}^{0.936}}{(J_{Gr} + J_{1r})^{0.474}}$
2	$\varphi_r = 0.16\left(\frac{J_G^2}{J_{1r}}\right)^{0.56}\left(1 + \frac{A_d}{A_r}\right)$
	$\varphi_d = 0.89\varphi r$
	$\varphi_r = 1.07Fr^{0.333}$
3	$Fr = \frac{J_G^2}{gD_r}$
4	$\varphi = 0.55J_{Gr}^{0.78}F^{0.2}D_r^{0.42}$
	$F = \frac{V_{1s}}{V_1}$
5	$\varphi_r = 0.203\frac{Fr_*^{0.31}}{Mo^{0.012}}\left(\frac{J_{Gr}}{J_{1r}} \cdot \frac{A_r}{A_d}\right)^{0.74}$
	$Mo = \frac{g(\rho_1 - \rho_G)}{\sigma_1\rho_1^2} \cdot K^4\left(\frac{8J_{1r}}{D_r}\right)^{4(n-1)}\left(\frac{3n + 1}{4n}\right)^{4n}$
	$Fr_* = \frac{(J_{1r} + J_{Gr})^2}{gD_r}$
6	$\varphi_d = 0.997\varphi_r$
7	$\varphi_r = 0.16\left(\frac{J_{Gr}}{J_1}\right)^{0.56}\left(1 + \frac{A_d}{A_r}\right)$

Appendix D – Less common dimensionless groups

Dimensionless groups relevant in bioreactor design as in (Chisti, Mass transfer, 1999)

Group	Definition	Physical significance
Bond number, *Bo*	$\frac{g d_p^2 \Delta\rho}{\sigma_L}$	$\frac{\text{Gravity force}}{\text{Surface tension force}}$
Peclet number, *Pe*	$\frac{U_L L}{D_L}$	$\frac{\text{Bulk mass transport}}{\text{Diffusional mass transport}}$
Poiseuille number, *Ps*	$\frac{\mu_L U_p}{\rho_L g d_p^2 \Delta\rho}$	$\frac{\text{Viscous force}}{\text{Gravity force}}$
Power number, *Po*	$\frac{P}{\rho_L N^3 d_i^5}$	
Rayleigh number, *Ra*	$Gr \times Sc$	
Stanton number, *St*	$\frac{Sh}{Re \times Sc}$	
Weber number, *We*	$\left(\frac{U_p^2 \rho_L d_p}{\sigma_L}\right)^{1/2}$	$\frac{\text{Inertial force}}{\text{Surface tension force}}$

Alternative formulations of cardinal dimensionless numbers (Chisti, Mass transfer, 1999)

Group	Alternative expressions
Reynolds number, *Re*	$\frac{\rho_L U_L d}{\mu_L}$ (in pipes and channels)
	$\frac{\rho_L N d_i^2}{\mu_L}$ (stirred tanks)
	$\frac{\rho_L U_L L}{\mu_L}$ (flow past a plate)
	$\frac{\rho_L U_L d_p}{(1-\phi)\mu_L}$ (flow past particles in packed beds)
Sherwood number, *Sh*	$\frac{k_L d}{D_L}$ (d may be diameter of a particle, flow channel, plate, etc.)
Froude number, *Fr*	$\frac{U_L^2}{g h_L}$, or $\frac{U_G^2}{g h_L}$ (in bubble columns and airlift fermenters)
	$\frac{N d_i^2}{g h_L}$, or $\frac{N^2 d_i}{g}$ (in stirred tanks)
Peclet number, *Pe*	$\frac{U_L h_L}{D_L}$, or $\frac{U_B d_B}{D_L}$ (in bubble columns and airlift fermenters)
	$\frac{N d_i^2}{D_L}$ (in stirred tanks)

Appendix E – Mass transfer coefficient correlations for ALRs

Examples of gas-liquid volumetric mass transfer coefficient for ALRs (Chisti, 1999)

Configuration	Equation	Ranges
Concentric-tube internal-loop vessels (annulus sparged)	$\frac{k_L a_D \sigma_L}{D_L g \rho_L} = 2.25\left(\frac{\mu_L}{\rho_L D_L}\right)^{0.500}\left(\frac{\rho_L \sigma_L^3}{g\mu_L^4}\right)^{0.136} \times \left(\frac{d_H}{d_T}\right)^{-0.0905} \varepsilon_G^{1.26}$ Average estimation error was 12% for 175 measurements Koide et al. (61)	Newtonian media, $3.71 \times 10^2 \le \mu_L/\rho_L D_L \le 6.00 \times 10^4$, $1.18 \times 10^6 \le \rho_L \sigma_L^3/g\mu_L^4 \le 5.93 \times 10^{10}$, $0.471 \le d_i/d_T \le 0.743$, $7.14 \times 10^{-3} \le d_H/d_o \le 2.86 \times 10^{-2}$, $0.0302 \le \varepsilon_G \le 0.305$, $6 \le$ aspect ratio ≤ 15, $0.52 \le A_d/A_r \le 1.23$
Concentric-tube internal-loop vessels (draft-tube sparged)	$\frac{k_L a_D d_T^2}{D_L} = 2.66\left(\frac{\mu_L}{\rho_L D_L}\right)^{0.500}\left(\frac{g d_T^2 \rho_L}{\sigma_L}\right)^{0.715}\left(\frac{g d_T^3 \rho_L^2}{\mu_L^2}\right)^{0.251} \times \left(\frac{d_i}{d_T}\right)^{-0.429} \varepsilon_G^{1.34}$ Koide et al. (62)	Newtonian media, $\rho_L = 997 - 1182$ kg m^{-3}, $\mu_L = (0.894 - 17.0) \times 10^{-3}$ Pa s, $\sigma_L = (51.7 - 73.0) \times 10^{-3}$ N m^{-1}, $D_L = (0.145 - 2.42) \times 10^{-9}$ m^2 s^{-1}, $A_r/A_d = 0.3$–0.8, aspect ratio = 6–15
External-loop reactor	$\frac{k_L a_L L}{U_{Lr}} = 14.5\left(\frac{U_{Gr}}{U_{Lr}}\right)^{0.83} Pe^{-0.6}$ Pe value is for the entire loop Verlaan et al. (63)	Air and water in 0.165 m^3 reactor, aspect ratio = 16 (based on riser), $A_r/A_d = 4$, no gas in downcomer, $0.01 \le U_{Gr}$(m s^{-1}) ≤ 0.14, $Pe = U_L L/E_L$, $Pe = 40$–60 (increased with gas flow rate)
A bubble column and a draft-tube sparged concentric-tube airlift vessel	$\frac{k_L a_L d_T^2}{D_L} = 0.018\left(\frac{K(2{,}800U_G)^{n-1}}{\rho_L D_L}\right)^{0.5}\left(\frac{g d_T^2 \rho_L}{\sigma L}\right)^{0.20} \times \left(\frac{U_G}{\sqrt{g d_T}}\right)^{0.51}\left(\frac{g d_T^3 \rho_L^2}{[K(2{,}800U_G)^{n-1}]^2}\right)^{0.62} \times (1 + 0.12\mathrm{Wi})^{-1}$ Suh et al. (64)	Non-Newtonian xanthan fermentation broths of the obligate aerobic bacterium *Xanthomonas campestris*. Bubble column (0.05 m^3, aspect ratio ≈ 19); airlift bioreactor (1.2 m^3, $A_r/A_d = 1$, aspect ratio ≈ 12)
Concentric-tube internal-loop (draft-tube sparged)	$k_L a_L = 3.43 \times 10^{-2} U_{Gr}^{0.524} \mu_{ap}^{-0.255}$ μ_{ap} was calculated by assuming the shear rate to equal $5{,}000 \times U_{Gr}$ for $U_{Gr} \ge 0.04$ m s^{-1}, or $1{,}000 \times (U_{Gr})^{0.5}$ for $U_{Gr} < 0.04$ m s^{-1} Li et al. (60)	Aqueous carboxymethyl cellulose ($K = 0.286$–11.5 Pa s^n; $n = 0.441$–0.617), 0.055 m^3 vessel, $A_r/A_d = 0.618$, aspect ratio ~ 26. $0.020 \le \mu_{ap}$ (Pa s) ≤ 0.85
Concentric-tube internal-loop reactors (draft-tube sparged)	$\frac{k_L a_D d_T^2}{D_L} = 2.66\left(\frac{\mu_L}{\rho_L D_L}\right)^{0.500}\left(\frac{g d_T^2 \rho_L}{\sigma_L}\right)^{0.715}\left(\frac{g d_T^3 \rho_L^2}{\mu_L^2}\right)^{0.251} \times \left(\frac{d_i}{d_T}\right)^{-0.429} \varepsilon_G^{1.34}\left(1 + 0.099\left(\frac{C_S}{\rho_S}\right)^{0.069}\right) \times \left(\frac{\rho_L \sigma_L^3}{g\mu_L^4}\right)^{0.023}\left(\frac{U_t}{U_G}\right)^{0.046}\right)^{-1}$ Average estimation error was within 17% for 383 measurements Koide et al. (62)	Suspensions of glass or bronze spheres, $3.71 \times 10^2 \le \mu_L/\rho_L D_L \le 9.92 \times 10^4$, $1.36 \times 10^3 \le g d_T^2 \rho_L/\sigma_L \le 1.22 \times 10^4$, $1.29 \times 10^8 \le g d_T^3 \rho_L^2/\mu_L^2 \le 1.26 \times 10^{11}$, $0.471 \le d_i/d_T \le 0.743$, $3.99 \times 10^{-2} \le \varepsilon_G \le 2.73 \times 10^{-1}$, $1.69 \times 10^{-11} \le g\mu_L^4/\rho_L \sigma_L^3 \le 2.55 \times 10^{-6}$, $0 \le C_S/\rho_S \le 8.00 \times 10^{-2}$, $1.17 \times 10^{-2} \le U_t/U_G \le 0.844$, $A_r/A_d =$ 0.3–0.8, aspect ratio = 6–15
Concentric-tube internal-loop reactors (draft-tube sparged)	$\frac{k_L a_D d_T^2}{D_L} = \frac{4.04}{1 + 2\varphi_S^{1.3}}\left(\frac{\mu_L}{\rho_L D_L}\right)^{0.5}\left(\frac{g d_T^2 \rho_L}{\sigma_L}\right)^{0.67}\left(\frac{g d_T^3 \rho_L^2}{\mu_L^2}\right)^{0.26} \times \left(\frac{d_i}{d_T}\right)^{-0.047} \varepsilon_G^{1.34}$ Average error of estimation was 14% for 260 data; U_G is based on the diameter d_T of the outer column; the $k_L a_D$ values in water and salt solutions were similar Koide et al. (65)	Suspensions of relatively low-density calcium alginate beads in water, aqueous glycerol, and aqueous salt solutions (0.10 or 0.27 kmol m^{-3} barium chloride; 0.4 kmol m^{-3} sodium sulfate), 0–20% v/v solids, 1.88–3.98-mm bead diameter, $A_r/A_d = 0.3$–1.2, aspect ratios = 6–16, $\mu_L = (0.894$–$12.5) \times 10^{-3}$ Pa s, D_L of oxygen = $(0.194$–$2.42) \times 10^{-9}$ m^2 s^{-1}, $3.71 \times 10^2 \le \mu_L/D_L\rho_L \le 5.52 \times 10^4$, $2.66 \times 10^3 \le \rho_L d_T^2 g/\sigma_L \le 1.22 \times 10^4$, $2.35 \times 10^8 \le \rho_L^2 d_H^3 g/\mu_L^2 \le 3.29 \times 10^{11}$, $0.471 \le d_i/d_T \le 0.743$, $1.69 \times 10^{-11} \le Mo \le 6.67 \times 10^{-7}$, $3.79 \times 10^{-2} \le \varepsilon_G \le 2.24 \times 10^{-1}$, $0 \le \varphi_S \le 0.2$; where the Morton number is $Mo = \frac{g(\rho_L - \rho_G)}{\sigma_L^3 \rho_L^2} K^4\left(\frac{8U_{Lr}}{d_r}\right)^{4(n-1)}\left(\frac{3n+1}{4n}\right)^{4n}$