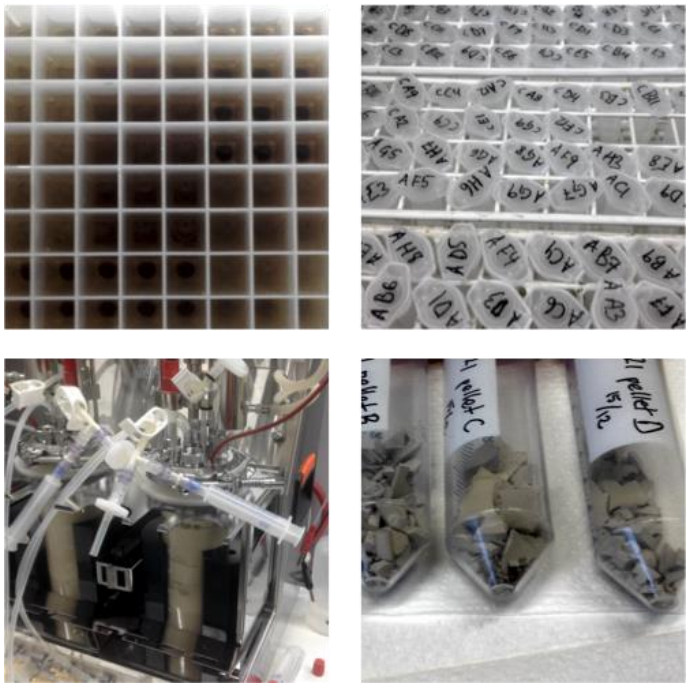
Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and
Agricultural Sciences

Fodder Yeast and Biogas Production: A Fruitful Symbiosis?

– Optimization of a Single-Cell Protein Process and Examination of its Impact on Biogas Output

Jonas Ohlsson



Department of Microbiology
Master's Thesis • 30 hec • Second cycle, A2E
Agriculture Program – Food Science • Examensarbete/Sveriges lantbruksuniversitet,
Institutionen för mikrobiologi, 2016:5 • ISSN 1101-8151
Uppsala 2016

Fodder Yeast and Biogas Production: A Fruitful Symbiosis? - Optimization of a Single-Cell Protein Process and Examination of its Impact on Biogas Output

Jonas Ohlsson

Supervisor: Su-lin Hedén, Swedish University of Agricultural Sciences, Department of Microbiology

Assistant Supervisor: Matilda Olstorpe, Swedish University of Agricultural Sciences, Department of Microbiology

Examiner: Volkmar Passoth, Swedish University of Agricultural Sciences, Department of Microbiology

Credits: 30 hec
Level: Second cycle, A2E
Course title: Independent project/degree project in Food Science – Master's thesis
Course code: EX0425
Programme/education: Agriculture Programme – Food Science

Place of publication: Uppsala
Year of publication: 2016
Cover picture: Jonas Ohlsson
Title of series: Examensarbete/Sveriges lantbruksuniversitet, Institutionen för mikrobiologi **No:** 2016:5
ISSN: 1101-8151
Online publication: http://stud.epsilon.slu.se

Keywords: fodder yeast, biogas, nutrient recycling, *Wickerhamomyces anomalus*, *Pichia kudriavzevii*, *Blastobotrys adeninivorans*

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Uppsala BioCenter
Department of Microbiology

Abstract

Due to a growing world population and strained wild fish stocks, aquaculture is expected to provide a large part of the increased global demand for animal protein, as the majority of wild fish stocks are already being fished at or above their sustainable capacity. However, fish feed often contains a significant amount of fish meal, produced from wild-caught fish. Thus, increasing farmed fish production may not be sustainable as long as fish meal remains a major ingredient.

Microbial biomass, commonly known as single-cell protein (SCP), can replace fish meal in feed formulations. The financial viability of SCP production is dependent on the availability of cheap growth media. Biogas substrates, often mixtures of waste derived from food industry, agriculture, and households, could be ideal media due to their low cost and ubiquitous availability.

The aims of this thesis were: first, to investigate whether yeast SCP can be produced on a biogas substrate consisting of household and agricultural waste; second, to screen several yeast strains and growth conditions for optimal biomass production; and third, to evaluate the effects on biogas production if part of input substrate stream is diverted into SCP production prior to further digestion in the biogas reactor.

Several yeast strains were screened for biomass yield at several pH levels and temperatures. After screening, best-performing strains were cultivated on biogas substrate in continuously-stirred bioreactors, analyzing the resultant biomass. Strains were *Wickerhamomyces anomalus* J121, *Pichia kudriavzevii* J550, and *Blastobotrys adeninivorans* J564. Harvested biomass ranged from 7.0–14.8 g dry matter per l substrate, and protein contents were 22.6–32.7 %. Levels of the amino acid lysine were high in all biomass samples, which is important for replacing fish meal in feed formulations.

Supernatants from the bioreactor fermentations were combined with whole biogas substrate, and the effects on biogas production were evaluated, as methane produced per g of volatile solids, using a batch methane potential assay. At the highest inclusion levels (1:1 control/supernatant (w/w)) *P. kudriavzevii* provided an increased output compared to *W. anomalus* (25 %, $p = 0.04$). *P. kudriavzevii* provided a 24 % increase compared to control, but results were not significant ($p = 0.06$). Filtered, untreated supernatant provided the largest increase in biogas production compared both to control substrate (30 %, $p = 0.02$) and to *W. anomalus* (31 %, $p = 0.01$).

Sammanfattning

I takt med en ständigt växande världsbefolkning förväntas efterfrågan på animaliskt protein öka. Akvakultur, eller fiskodling, kommer sannolikt att bidra till en stor del av den förväntat ökande produktionen. Produktionen från majoriteten av vilda fiskbestånd beräknas inte kunna öka ytterligare, och en stor andel klassas redan som överfiskade. Då fiskmjöl, som framställs från vildfångad fisk, utgör en viktig ingrediens i fiskfoder är det inte säkert att en utökad produktion av odlad fisk är hållbar, och alternativa proteinkällor är därför efterfrågade.

En sådan proteinkälla utgörs av mikrobiell biomassa, eller singelcellprotein (SCP). För storskalig produktion av SCP krävs tillgång till billigt tillväxtmedium. Biogassubstrat, ofta en blandning av avfall från livsmedels- och jordbrukssektorn samt hushåll, skulle kunna utgöra ett idealt sådant medium p.g.a. dess låga kostnad samt höga tillgänglighet.

Syftet med detta arbete var tredelat: att undersöka om SCP från jäst kan produceras på biogassubstrat bestående av hushålls- och jordbruksavfall; vidare att utvärdera flera jäststammar samt odlingsbetingelser för optimal tillväxt; och slutligen att utvärdera effekten på biogasproduktion om jäst odlas på en del av biogassubstratet före dess introduktion i biogasreaktorn.

Ett antal jäststammar utvärderades för biomasseproduktion vid flera temperaturer samt pH-nivåer. De mest högproducerande stammarna odlades sedan på biogassubstrat i fermentorer, och den producerade biomassan analyserades. Dessa var *Wickerhamomyces anomalus* J121, *Pichia kudriavzevii* J550 och *Blastobotrys adeninivorans* J564. Fermentationerna resulterade i slutlig biomassa på 7.0–14.8 g per l substrat, och innehöll 22.6–32.7 % protein.

Supernatanterna från odlingarna blandades med orört biogassubstrat och utvärderades för metanproduktion, som mängd metan per g glödförlust, i ett batchförsök. Vid den högsta graden inblandning (obehandlat substrat och supernatant i ett 1:1-förhållande baserat på vikt) producerade prover baserade på *P. kudriavzevii* 25 % mer biogas jämfört med motsvarande från *W. anomalus* ($p = 0.04$). En 24 %-ig ökning kunde ses då supernatant från *P. kudriavzevii* J550 jämfördes med kontrollsubstrat, men detta resultat uppnådde inte statistisk signifikans ($p = 0.06$). Den största ökningen av biogasproduktion sågs vid tillsats av obehandlat, filtrerat substrat, som presterade bättre än både obehandlad kontroll (30 %), $p = 0.02$) och *W. anomalus* (31 %, $p = 0.01$).

Contents

List of Tables

List of Figures

Acronyms and abbreviations

AA	Amino acid
AD	Anaerobic digestion
BMP	Biomethane potential
CFU	Colony forming unit
CL	Crude lipid
CP	Crude protein
CSTR	Continuously stirred tank reactor
EAA	Essential amino acid
FM	Fish meal
FO	Fish oil
GHG	Greenhouse gas
IHT	Inter-species hydrogen transfer
OD_{600}	Optical density at 600 nm
RNA	Ribonucleic acid
QPS	Qualified presumption of safety
SCP	Single-cell protein
TS	Total solids
VS	Volatile solids

1 Introduction

With an ever increasing world population, resource scarcity is expected to become a global problem. In 2050, the world population is expected to reach 9.6 billion (FAO, 2014), which will likely put a strain on the world's food and energy supplies. To meet the heightened demand, efforts towards increasing food and energy outputs are required. Increasing these outputs does come with its own cost however, as the world's supply of natural resources (including, but not limited to, fossil fuels and phosphorus) are further diminished while promoting higher rates of greenhouse gas (GHG) emissions by the burning of fossil fuels.

Aquaculture, and especially the practice of fish and shellfish farming, has supplied a substantial portion of the increased demand for animal protein during the last few decades, and is expected to continue doing so as the world's population grows larger. It is the fastest growing primary animal food producing sector, exhibiting an average annual rate of expansion of 6.2 % in the years 2000–2012 (FAO, 2014). Although farmed fish and shellfish exhibit good feed conversion ratios compared to many other farmed animals, a large part of aquaculture is still dependent on decreasing marine fish stocks

for feed. Dwindling supplies and increasing prices mean that alternatives to fish meal (FM), such as soya meal, are being used as replacements.

Another FM alternative is microbially produced biomass, a novel source of calories commonly known as single-cell protein (SCP; Matelbs and Tannenbaum (1968)). Although not entirely comprised of protein, this macronutrient has usually been the focus of such products. These products may be marketed either for human consumption, or as ingredients of animal feed. SCP has several benefits compared to other feedstocks: large amounts of biomass can be manufactured in short time; organisms used for SCP production normally have low nutrient requirements; often the substrates used are low-value commodities, or side streams and waste products from other manufacturing processes.

To reduce GHG emissions and promote energy independence and energy security as fossil fuels become scarcer, methods for bioenergy production are the focus of much research. Biogas production is one such method, whereby waste products are converted into energy-rich methane (CH_4) and nutrient-rich fertilizer (digestate) in an anaerobic digestion (AD) process.

Nutrient recycling and efficient use and generation of energy are important factors for sustaining the world in light of what's been discussed above, and their importance will increase with resource scarcity. Feeding a growing world population using fewer resources will likely become one of the most pressing issues of this century. The aim of this thesis is to investigate a combined SCP and biogas production using the same substrate, and assess whether it represents a novel way of increasing resource utilization and nutrient recycling.

1.1 Aquaculture and fish farming

Aquaculture refers to the cultivation of water-living creatures such as fishes and mollusks, but also aquatic plants. However, the focus on aquaculture in this thesis refers exclusively to finfish, as this is the group of aquaculture organisms that are the intended recipients of the SCP product being developed.

Since the beginning of the 1980s, the increase in world fish production is entirely due to farmed fish (FAO, 2014; see Fig. 1). If current trends continue, it will become the largest animal food producing sector in a few years. In Fig. 2, both fish and livestock (including chicken) production are displayed for comparison (FAO (2014, 2016).

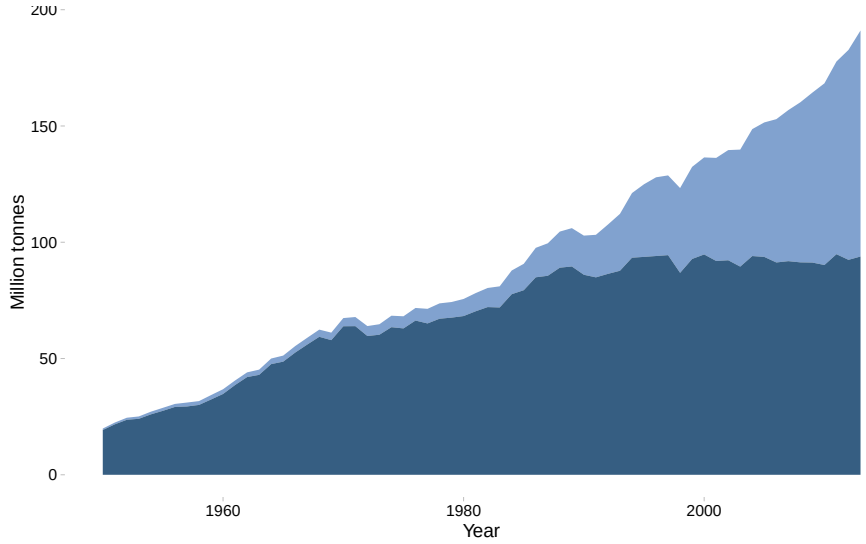


Figure 1: World fisheries and aquaculture production by year, 1950–2013 (FAO, 2014). Legend: Fisheries production, dark blue; aquaculture production, light blue.

1.1.1 Fish farming in Europe and the Nordic countries

Fish farming in Europe is dominated by Norwegian production, which amounted to 1.4 million tonnes in 2014. This is almost eight-fold greater than Europe's next-largest producer, United Kingdom, and twice as much as all EU countries combined. Except for Norway and the Faroe Islands (86,000 tonnes in 2014), the Nordic countries do not have well-developed fish farming industries (FEAP Secretariat, 2015).

Species farmed in Norway and the Faroe Islands are mainly Atlantic salmon (*Salmo salar*), with rainbow trout (*Oncorhynchus mykiss*) making up a small amount (FEAP Secretariat, 2015). Both fish are carnivorous salmonid species.

1.1.2 Feed composition and ingredients

Fish meal (FM) and fish oil (FO) are major ingredients in feeds for carnivorous fish. For salmon feed, the total amount of combined FM and FO in feed formulations ranges from 35–65 % (FAO, 2011). This practice cannot be considered sustainable, since the demand from aquaculture will likely increase pressure on already taxed wild fish stocks. The use of captured fish, containing high-value protein and fatty acids, for use in farmed fish feed competes directly with the supply of these nutrients to the human food

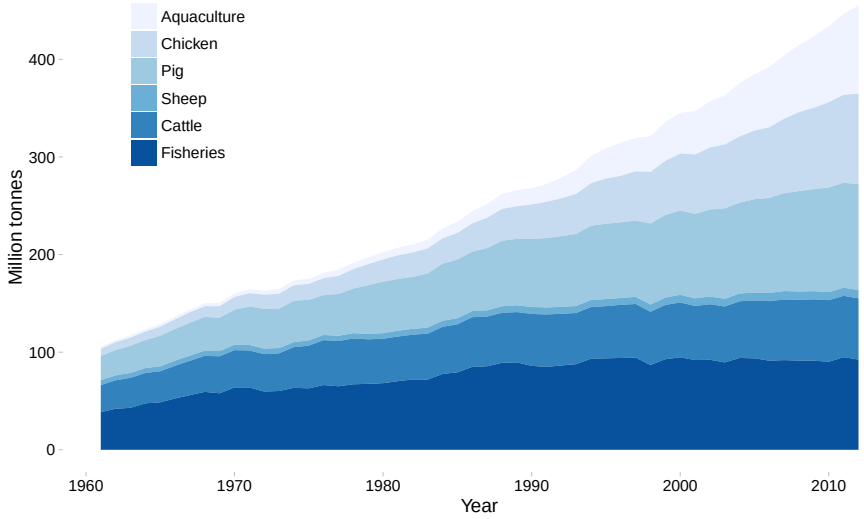


Figure 2: World fisheries, aquaculture, and livestock production by year, 1961–2012. Data from FAO (2014, 2016).

chain. Thus, as these resources become scarcer, a competition between fish farmers and people living in developing countries may occur, because fish farmers operating in more affluent regions may be able to pay a higher price for these resources (Tacon and Metian, 2009).

Although the inclusion rate of FM and FO in fish feed formulations is decreasing (Naylor et al., 2009), alternative feed sources are required to sustain an ever increasing fish farming industry in the wake of a 9.6-billion world population. Plant-based sources could be more sustainable, but they could also compete for human nutritive interests and carry with them additional issues, ranging from deforestation to poor amino acid profiles, and are not likely to completely replace FM and FO in feed formulations.

1.2 Single-cell protein

1.2.1 Production of single-cell protein

Microorganisms are in many ways extremely well suited for protein production, compared to many other organisms that are used today in agriculture (such as cows and soya beans). They often have a very good feed conversion ratio, and grow quickly; they can grow on waste products of many kinds; they do not require much in the form of resources such as land, energy, etc.; and the facilities used to produce them can basically be deployed anywhere.

The interest in using biomass of microbial origin emerged during the 20th century, spurred by declining availability of animal food products. Although originally seen as a way of providing cheap human nutrition, either during wartime or in impoverished countries, the development of such products has so far not seen large-scale deployments. Rather, the primary focus of SCP production is now animal fodder, and only a few mainstream examples of products sold for human consumption exist (Quorn™, Section 1.2.2; Waites et al. (2009)).

The production of SCP is carried out in a *fermenter*, also known as a *bioreactor*. The use of a fermenter allows strict control of production parameters such as pH, stirring and aeration, nutrient levels, temperature, etc., as well as providing an aseptic environment for consistent quality. Depending on the purpose of the fermentation, different fermenter designs and monitoring parameters are chosen. For SCP production, a high degree of aeration and stirring, strict pH control and an aseptic environment are common features found in most fermenters used.

Laboratory-scale fermenters are often continuously stirred tank reactors (CSTRs), where agitation is carried out using impellers and baffles are used to increase turbulence in the reactor. In larger-scale operations, airlift fermenters are more commonly used. Airlift fermenters do not have impellers, but instead inject compressed air or other gases into the bottom of the fermenter to accomplish mixing. This fermenter design is more energy efficient than a CSTR, and also reduces shear forces which may damage the cells.

Although the fermenter is arguably the most important piece of equipment for SCP production, it is also important that the product can be separated from the medium in an efficient manner. Depending on the organism, different systems for separation are used. For example, some organisms may float on the surface as foam, others sediment, and still others form filaments. Filtration and centrifugation systems are commonly employed. Flocculation can simplify separation, and can be induced in a number of ways. A few examples from commercial facilities are mentioned in Section 1.2.2.

A major concern for the viability of an SCP producing operation is the availability of cheap substrate. A number of production facilities listed by Goldberg (1985) have failed in the long term due to changes in market price or availability of the intended substrate. Notable examples include processes based on the use of crude oil fractions, which relied on low prices, and those using spent sulfite liquor (a by-product of the paper industry), a substrate that became unavailable as new paper production methods were implemented.

1.2.2 Commercial examples

Perhaps the best-known example of an SCP product is the meat substitute Quorn™, which is produced from the mycelium of *Fusarium venenatum* ATCC 20334 (Waites et al., 2009). The development of this product began in the 1960s, and took approximately 20 years of research and development to bring to market. Initially, food-grade industrial effluents were used for the culture medium, but today the process uses a completely defined medium (Wiebe, 2004).

Development of Quorn™ took place in several scale-up steps, from a 10-l fermenter in 1967 to the dual 150,000-l reactors used today (Goldberg, 1985; Wiebe, 2004), and included the screening of over 3,000 fungal strains. Today, the yield of the process is approximately 0.5 g product for 1 g glucose (dry weight). The use of a filamentous fungus for the process is beneficial both by providing the product with a meat-like texture, and by decreasing the cost of separation compared to single-cell organisms (Goldberg, 1985; Waites et al., 2009).

Although initial motivations for SCP production were largely based on feeding a growing population directly, only a small fraction of large-scale SCP production has been marketed for this purpose, with the bulk of the output being sold as animal feed. *Pruteen* is an example of one such SCP product, which was produced by Imperial Chemical Industries, Ltd. in the United Kingdom (Goldberg, 1985). This product consisted of dried cells of the bacterium *Methylophilus methylotrophus*, and methanol was used as the carbon input in its production.

Pruteen was produced in the world's largest aerobic aseptic fermenter, with a capacity of 1,500 m^3 (Goldberg, 1985). In the production of Pruteen, flotation and flocculation were used to separate the product from the medium, and the medium was recirculated into the reactor after separation. Although well tolerated as a feedstock (Braude and Rhodes, 1977), the cost of production made Pruteen uncompetitive in the market, and production was later discontinued.

1.2.3 Microbial fish feed

SCP products have also been used in fish feed formulations. Fungal SCP requires an ribonucleic acid (RNA) reduction step when intended for human consumption. In humans and other simian primates (Goldberg, 1985), purine-containing compounds such as RNA are metabolized into uric acid, which may accumulate and lead to health problems. However, at least some fish species are able to degrade uric acid, thereby avoiding the toxic effects of consuming high-purine feedstuffs (Rumsey et al., 1992). Thus, an SCP

production facility producing feed for these fish species does not require an RNA reduction step, reducing energy requirements and capital costs of the production facility. Furthermore, SCP-based feedstuffs do not compete with human nutritional interests as long as they are not grown on substrates normally used for food.

Yeast feeding experiments on fish have yielded mixed results concerning effects on growth, ranging from large negative to large positive effects (Eliasson, 2015). The heterogeneous results may be largely due to differences in fish species and yeast strains utilized, as well as digestibility of the feed. Fish growth rate seems to depend on the proportion of FM being replaced by yeast SCP, with lower proportions of SCP generally being more conducive to growth. That is, replacing too much FM with SCP seems to have a negative influence on growth.

Fish require ten essential amino acids (EAA) to be provided in the feed (Wilson and Halver, 1986). For these EAA, yeast-based SCP generally exhibits an amino acid profile close to that of FM (Vidaković, 2015), although levels of methionine and arginine are slightly lower. Altogether, yeast-based SCP feedstuffs seem like good candidates for at least partly replacing FM in fish feed.

1.3 The anaerobic digestion process

1.3.1 Overview

Biogas, a methane-rich gas mixture, is formed by AD processing of a wide variety of substrates including waste products from households, industries and agriculture, as well as crops grown specifically for biogas production. The AD process can be divided into four distinct but co-occurring phases: hydrolysis, fermentation, acetogenesis, and methanogenesis (Angelidaki et al. (2011); Fig. 3).

The hydrolytic phase involves enzymatic degradation of complex polymers such as polysaccharides and proteins, as well as simpler compounds including triglycerides and disaccharides, into their monomeric constituents. The resulting compounds are then fermented, generating mainly acetate, ethanol, and butyrate (Angelidaki et al., 2011), as well as hydrogen gas (H_2) from certain compounds. In the case of amino acids, ammonia (NH_3) is also formed.

In the acetogenic phase, acetate is formed either by oxidation (*e.g.* from butyrate or ethanol), or by reduction of CO_2 to acetyl-CoA. Oxidation is carried out using H^+ as an electron acceptor, leading to the formation of H_2 gas. This reaction is energetically unfavorable and requires that the

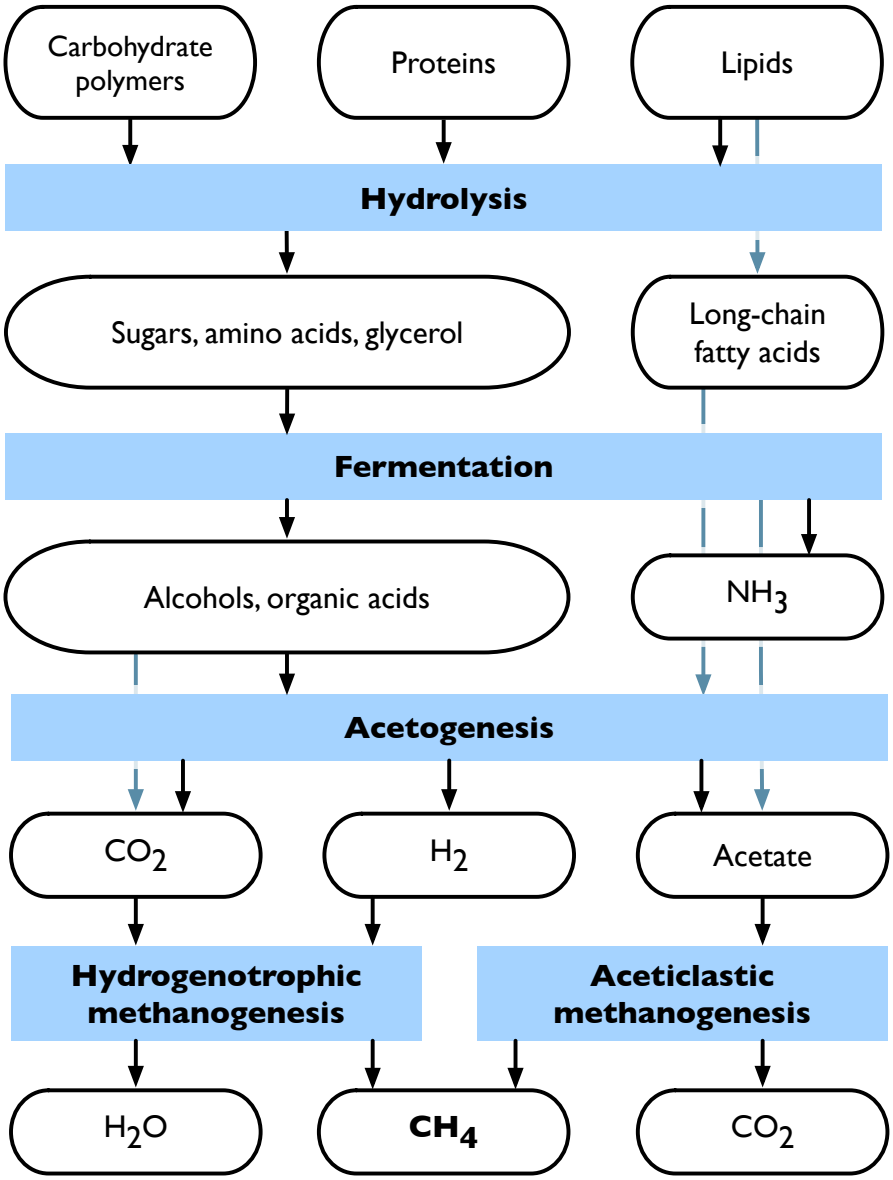


Figure 3: Overview of the anaerobic digestion process. Adapted from Angelidaki et al. (2011); Weiland (2009).

concentration of end products be kept low, thus it must be coupled to the methanogenic process via inter-species hydrogen transfer (IHT).

Methanogenesis can be carried out in three different modes (Angeli-

daki et al., 2011): aceticlastic, forming CH_4 and CO_2 from the cleavage of acetate; hydrogenotrophic, where CO_2 is reduced into CH_4 using H_2 as electron donor (thus consuming H_2 produced in the acetogenic phase); and methylotrophic, which uses compounds such as methanol (CH_3OH) and methylamine (CH_3NH_2) as substrates. In a typical biogas production facility, the first two modes are the main contributors to methane synthesis.

1.3.2 Evaluating methane potential

At least partly due to the complex nature of the AD process, estimating the methane production from a given substrate needs to be done on a per-substrate, per-inoculum basis. Many factors influence the potential output of methane, including but not limited to particle size, oxidation state, amount of volatile solids (VS), C:N ratio, makeup of AD microbial community, presence of micronutrients, and presence of toxic contaminants (Angelidaki et al., 2011; Esposito et al., 2012).

One of the simplest methods of methane potential estimation is known as the biomethane potential (BMP) assay. In this assay, substrate and inoculum are mixed in bottles according to a pre-determined ratio based on the amount of VS in each. Bottles are then filled with water to a predetermined volume to ensure a consistent headspace volume, and are capped. From a hole in the cap, gas output is transferred via a CO_2-capture device (i.e. *upgraded*) to a measuring apparatus. As biogas is comprised mainly of CH_4 and CO_2, the measured gas is considered to be pure CH_4. Although no standard protocol exists for BMP assays, Angelidaki et al. (2009) is a widely cited protocol.

2 Overview and common methods

2.1 Overview of experiments

The work outlined in this thesis is multi-faceted, and this section outlines the experimental work to give an overview to the reader. The aim of this work was to investigate optimal yeast strains and growth conditions on biogas substrate; to assess biomass output and growth kinetics in a lab-scale bioreactor; and finally to evaluate the impact on biogas output of the substrate post-fermentation.

2.2 Common materials and methods

2.2.1 Biogas substrate and inoculum

The substrate used for the experiments in this section came from a biogas plant in Sweden, and consisted of source-separated household waste and

manure. A chemical characterization of the substrate was performed at Agrilab AB, Uppsala, Sweden (see Table 1). The pH of the substrate was approximately 5. The substrate was heat treated at the biogas plant for 90 min at 70 °C, but was likely contaminated during sampling at the biogas facility.

Table 1: Chemical characterization of the biogas substrate

Parameter	Value
Total solids (%)	7.8
Volatile solids (% of TS)	86.3
Total N (g/kg)	2.7
Organic N (g/kg)	2.2
NH_4^+-N (g/kg)	0.5
Total C (g/kg)	36.3
C/N ratio	13.3
Total P (g/kg)	0.37
Total K (g/kg)	1.37
Total Mg (g/kg)	0.24
Total Ca (g/kg)	1.77
Total Na (g/kg)	0.52
Total S (g/kg)	0.24

For the majority of the screening experiments, the substrate was filtered using an Asahi Rexeed-25A hemodialyzer (Asahi Kasei Medical Co., Ltd., Tokyo, Japan) connected to a peristaltic pump, ensuring that the pressure never exceeded 0.6 bar (see Fig. 4 for an illustration of the substrate before and after filtration). For the scale-up experiments, the substrate was further sterilized via vacuum filtration through a 0.2 µm sodium acetate filter (Nalgene Rapid-Flow, Thermo Fisher Scientific, Waltham, MA, USA).

For the methane potential assay, a fresh AD culture was acquired from the same biogas facility. The culture was stored at 37 °C until the methane potential assay could be initiated (approx. 7 days).

2.2.2 Culture media and chemicals

Culture media and solutions used for the experimental work in this thesis are outlined in Table 2. All media were autoclaved at 121 °C before use.

2.2.3 Yeast strains

A number of yeast strains had previously been identified as good candidates for use in this work, based on their growth performance and effect on

Figure 4: Biogas substrate before (left) and after filtration.

methane production on the spent substrate (Matilda Olstorpe & Simon Isaksson, unpublished data). These were *Wickerhamomyces anomalus* strains J121 and J379, organisms known for their metabolic versatility, robustness to different growth conditions, and biocontrol properties (Passoth et al., 2006; Schnürer and Jonsson, 2010); *Yarrowia lipolytica* J134, a yeast adept at digesting lipids and exhibiting a large secretory activity (Coelho et al., 2010); *Pichia kudriavzevii* J550, a very robust yeast known for its ability to grow well in the presence of inhibitory substances (Radecka et al., 2015); and *Blastobotrys adeninivorans* strains J562 and J564, yeasts well-known for being able to metabolize a wide variety of compounds (Middelhoven et al., 1991). See Table 3 for alternative strain numbers.

It is worth noting that *W. anomalus* J121 has been evaluated in fish feeding trials. Using a 1:1 blend of *W. anomalus* J121 and *Saccharomyces cerevisiae*, it was found that growth was not negatively impacted when replacing up to 40 % of FM in the diets (Vidaković, 2015).

Yeast strains were taken from 50 % glycerol stocks kept at −80 °C at

Table 2: Culture media and solutions used for the experiments

Medium	Constituents per 1 l deionized water
Wickerham's Yeast and Mold agar with Chloramphenicol (YMC)	3 g yeast extract (BD, Le Pont-de-Claix, France) 3 g malt extract (BD, Le Pont-de-Claix, France) 5 g bacterial peptone (BD, Le Pont-de-Claix, France) 10 g D-glucose (Merck, Darmstadt, Germany) 100 mg chloramphenicol (Sigma-Aldrich, Steinheim, Germany) 18 g agar (BD, Le Pont-de-Claix, France)
Yeast extract–peptone–D-glucose (YPD) broth and agar	10 g yeast extract 20 g bacterial peptone 20 g D-glucose (20 g agar)
0.1 % peptone water	1 g bacterial peptone
0.9 % NaCl solution	9 g sodium chloride (Sigma-Aldrich, Steinheim, Germany)

Table 3: Yeast strains used in this thesis, with alternative strain numbers

Strain	Alternative strain number
W. anomalus J121	Local isolate
Y. lipolytica J134	CBS 6114
W. anomalus J379	VKM Y-160
P. kudriavzevii J550	CBS 2062
B. adeninivorans J562	CBS 8244
B. adeninivorans J564	CBS 7377

the Swedish University of Agricultural Sciences, Uppsala, Sweden, and subcultured onto YPD agar.

2.2.4 Yeast inoculum preparation

For all experiments, inoculum cultures were grown in YPD broth for approximately 24 hours in 125-ml baffled Erlenmeyer flasks (Thomson Ultra-Yield, Thomson Instrument Co., Carlsbad, CA, USA) at 30 °C on a rotary shaker set to 150 rpm. Cells were harvested by centrifugation at 3000 × *g* for 5 min, and washed using 0.9 % NaCl at the same centrifuge settings. Centrifugation was performed on a Beckman J26-XPi centrifuge (Beckman Coulter Inc., Brea, CA, USA), equipped with a Beckman JS-4.2 swinging-bucket rotor.

Harvested cells were resuspended in 0.9 % NaCl solution and OD_{600} was

measured on an Ultrospec 1100 pro UV/VIS spectrophotometer (Biochrom Ltd., Cambridge, UK).

2.2.5 Viable cell counts and biomass measurements

For viable cell counting, serial dilutions were made using 0.1 % peptone as the diluent. CFU counts were performed by dividing YMC agar plates into three sectors, with each sector representing one dilution factor. In each sector, five 10 µl droplets were inoculated, allowing the liquid to be absorbed by the agar before moving the plate. The plates were incubated at 30 °C for approx. 24 h, or until colonies were clearly visible.

For measuring biomass produced, 1.5-ml Eppendorf tubes were dried overnight at 105 °C to minimize plasticizer losses during drying. Tubes were marked and weighed. A sample was transferred into the tube, centrifuged at $6000 \times g$ for 5 min, and the supernatant discarded. The pellet was resuspended in 1 ml deionized water and centrifuged once more using the same settings, again discarding the supernatant. The tubes were then dried overnight at 105 °C, and weighed to calculate the dry weight of the biomass.

2.2.6 Bioreactor operation

Four 500-ml Infors HT Multifors CSTR bioreactors (Infors AG, Bottmingen, Switzerland) were used for the fermentations. For each reactor, 400 ml of substrate was inoculated to give an initial OD_{600} of 1.0. Reactor parameters were $pH = 7.00 \pm 0.10$, stirrer = 500 rpm, and $pO_2 = 0.2$. Aeration was maintained using stirrer speed, with a minimum of 200 rpm and a maximum of 1200 rpm. The reactors were outfitted with sampling ports designed to reduce contamination during sampling (Infors Super Safe Sampler, Infors AG, Bottmingen, Switzerland). pH was automatically adjusted on-line using 5 M NaOH and 3 M H_3PO_4.

For most reactor experiments, samples were collected every 3 hours during fermentation, including 0 h samples taken immediately following inoculation. For each time point and bioreactor, approx. 6 ml of the reactor contents were collected in sterile 15-ml Falcon tubes.

2.2.7 Chemical analyses

The chemical analyses of yeast pellets were performed at the VHC lab at the Swedish University of Agricultural Sciences, Uppsala, except for amino acid (AA) analyses which were performed at Eurofins Food & Agro Testing Sweden AB, Linköping. Supernatants and whole substrate were analyzed at Agrilab AB, Uppsala. All samples were stored at −20 °C until analyses could be performed.

Crude protein (CP) content was determined using the total nitrogen

Kjeldahl method, and CP was calculated as $N \times 6.25$ (Nordic Committee on Food Analysis, 2003). Crude lipid (CL) content was determined according to The Commission of the European Communities (1998). AA analyses were performed according to the SS-EN ISO 13903:2005 method.

2.2.8 Statistical analyses

All statistical analyses were performed using R version 3.2.2 (R Core Team, 2015). For assessing methane production, Tukey's Honest Significant Difference test was used.

3 Yeast screening

3.1 Materials and methods

For evaluating yeast biomass production under a number of different growth conditions, a miniaturized screening protocol was developed. Factors evaluated using this protocol included yeast strain, pH, and temperature. In short, 1-ml cultures were grown in deep-well 96-well plates and the contents of each well was evaluated for biomass output. This protocol allowed efficient screening of multiple growth conditions in a limited time. See Fig. 5 for an example plate layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Control	pH 6.55			Control	pH 6.76			Control	pH 7.81		
B	J121	pH 6.55			J121	pH 6.76			J121	pH 7.81		
C	J550	pH 6.55			J550	pH 6.76			J550	pH 7.81		
D	J562	pH 6.55			J562	pH 6.76			J562	pH 7.81		
E	J564	pH 6.55			J564	pH 6.76			J564	pH 7.81		
F	YPD control											
G												
H												

Figure 5: Example 96-well plate layout used in screening experiment.

Unless otherwise stated, all screening experiments were carried out in autoclaved 2.2-ml 96-well plates (Sarstedt MegaBlock 96 Well, Sarstedt AG & Co., Nümbrecht, Germany). The plates were covered with sandwich covers to ensure adequate aeration (μ-flask by Duetz, Applikon Biotechnology BV, Schiedam, The Netherlands). To each well, 900 μl of substrate was added, and inoculated to a final OD of 0.1, final volume 1000 μl. Incubation was performed on a rotary shaker, set to 300 rpm. Biomass was evaluated according to the methods described in Section 2.2.5. Each treatment was performed in four replicates. pH was adjusted using 5 M NaOH.

A pre-screening trial was performed to determine which pH levels were conducive to growth. Biogas substrate was pH-adjusted to several levels using 5 M NaOH and transferred to 125-ml baffled Erlenmeyer flasks (Thomson Ultra-Yield). The flasks were inoculated with fresh yeast cultures, and incubated at 30 °C on a rotary shaker for 24 h. For each pH level, multiple yeast strains were evaluated. Growth was assessed using light microscopy.

Based on the findings of these preliminary growth trials, an initial screening was performed to assess approximate pH optimum, best yeast candidates, and temperature. For choosing pH levels, the lowest level was chosen where all yeast grew acceptably. Two higher levels were chosen based on the amount of NaOH needed to adjust pH. At pH levels approaching 8, buffering capacity was low and it was therefore deemed unnecessary to assess growth at levels higher than this. Levels chosen for initial screening were 6.55, 6.76, and 7.81. Screening was performed at 25, 30, and 37 °C.

Further screening was subsequently done using the three best performing strains and a larger number of pH levels. The aim of this screening was to determine a pH level that ensured good growth for all three strains, for use in the methane potential assay (Section 4). The range of pH levels was chosen based on the results from the first screening trial, and was again based on the amount of NaOH added. Six pH levels ranging from 6.05–7.30 were assayed in this screening phase. Plates were incubated at 30 °C for 24 h.

3.2 Results

In total, the screening results are based on data from 654 cultures, including replicates. All yeast strains evaluated required some pH adjustment to grow well, with pH levels around 7 generally yielding the highest biomass production. Most yeast strains exhibited best growth at 30 °C, except for *B. adeninivorans* which produced the highest amount of biomass at 37 °C.

For most screening conditions, *B. adeninivorans* strain J564 outperformed strain J562, while exhibiting a similar response to growth conditions. This led to the exclusion of J562 from the later phases of screening. *W. anomalus* J379 also exhibited poor performance and was excluded at an early stage.

The results from the screening experiments are included in Appendix A. Unfortunately, not all results were easy to interpret, and comparisons between different screening runs were not always possible. This is discussed further in Section 6. Results obtained during this part of the experimental work served as a basis for bioreactor experiments described in Sections 4 and 5.

4 Effect on methane potential by yeast treatment of substrate

The aim of this trial was to assess whether results from a previous small trial (unpublished data) were reproducible. In the previous work, an increase in methane potential was observed when the supernatant of centrifuged substrate was yeast-treated and subsequently recombined with the solid material prior to AD.

Because the experimental design of the previous study was vulnerable to confounding factors, and due to the fact that the impact on methane potential is a crucial parameter in deciding whether implementing SCP production in a biogas setting is financially feasible, it was decided that a better-controlled study of this aspect was needed.

In short, yeast was cultured on filtered biogas substrate in CSTR bioreactors. The supernatant of the post-fermentation medium was combined with untreated substrate, and evaluated for biogas output in a BMP assay.

4.1 Materials and methods

Based on the results obtained in Section 3, as well as results from previous experiments, yeast strains *W. anomalus* J121, *P. kudriavzevii* J550, and *B. adeninivorans* J564 were chosen for this experiment. Fermentations were performed at 30 °C, pH 7.00 ± 0.10, with one strain per fermenter. A fourth fermenter containing uninoculated substrate was included as a control, running under the same parameters.

Fermentations for control substrate, *W. anomalus J121*, and *P. kudriavzevii* J550 lasted 23 h. Due to a problem with oxygen supply, the *B. adeninivorans* J564 reactor was allowed to run for an additional 8 h.

After fermentation, reactor contents were transferred into 1000-ml centrifuge bottles (Beckman Coulter Inc., Brea, CA, USA), noting the exact weights. Bottles were centrifuged at $3000 \times g$ for 10 min, and the supernatants were saved. Pellets were resuspended in deionized water, and centrifuged again using the same settings. The supernatants were discarded, and pellets were quantitatively transferred from the bottles and stored at −20 °C for further analysis. Centrifugation was performed on a Beckman J26-XPi centrifuge (Beckman Coulter Inc., Brea, CA, USA), equipped with a Beckman JS-4.2 swinging-bucket rotor.

Total solids (TS) and VS of whole substrate (pre-fermentation) and supernatants (post yeast-treatment) were determined by drying the substrates at 105 °C and incinerating at 550 °C, noting the weights after each step. TS was

calculated as the quotient of dry matter divided by initial weight. VS was then determined to be the difference between TS and ash content.

A methane potential assay was performed, comparing the effects of adding yeast treated supernatant to whole untreated biogas substrate (WS). Each treatment contained WS and fresh AD inoculum, contributing approximately 1.2 g VS and 3.6 g VS, respectively. One treatment contained only WS and AD. For the remaining treatments, supernatant was added in amounts corresponding to 10 %, 30 %, 50 %, and 100 % of the amount of WS by weight. In one treatment group, the supernatant was derived from the uninoculated control fermenter. This group was included to differentiate the effects of aeration, stirring and centrifugation of the filtered substrate from effects attributable to yeast growth. See Table 4 for an overview of the included treatment groups. Increases in VS due to supernatant additions were modest, at most 22 %, and it was assumed that the inoculum would not be overloaded by such slight differences. Inoculum and cellulose controls were included as process controls. Tap water was added to each bottle to a final volume of 400 ml, and each treatment was evaluated in triplicate.

Table 4: Overview of treatments included in the methane potential assay, comparing whole biogas substrate (WS) to WS with addition of supernatant from filtered substrate, with and without yeast treatment. X% is relative to the weight of WS added to each sample, and had values 10, 30, 50, and 100

Treatment	Description
WS	Untreated biogas substrate
WS + X% control	Same as WS, but with X% uninoculated supernatant added
WS + X% J121	Same as WS, but with X% supernatant of spent substrate from *W. anomalus* J121 fermentation added
WS + X% J550	Same as WS, but with X% supernatant of spent substrate from *P. kudriavzevii* J550 fermentation added
WS + X% J564	Same as WS, but with X% supernatant of spent substrate from *B. adeninivorans* J564 fermentation added

A BMP assay was performed using four AMPTS II Automatic Methane Potential Test Systems (BioProcess Control AB, Lund, Sweden). Gas volume measuring was done with a piston system, and gas was upgraded (i.e. CO_2 was removed) by flushing it through 7 M NaOH. Agitation was performed using stirrers attached directly to the bottles. The BMP assay was performed

at 37 °C, as this was the temperature used in the commercial digester from which the inoculum was derived. Bottles were flushed with N_2 gas prior to initiating the experiment. Specific methane potential was obtained by dividing the volume of methane produced by the actual amount of VS in each sample. The BMP assay was determined to be finished when all samples generated less than one gas emission reading per day.

4.2 Results

The growth curves for the yeasts grown in the CSTR bioreactors are presented in Fig. C1, Appendix C. For strains *W. anomalus* J121 and *P. kudriavzevii* J550, decreasing viable counts were observed in the later stages of fermentation. The decrease was accompanied by a reduction in OD_{600} values (data not shown). For *W. anomalus* J121, a sharp decrease in the rate of oxygen consumption was observed around the 12-h mark. Comparing the 9 h and 20 h samples using light microscopy revealed an apparent reduction in the number of cells (Fig. 6).

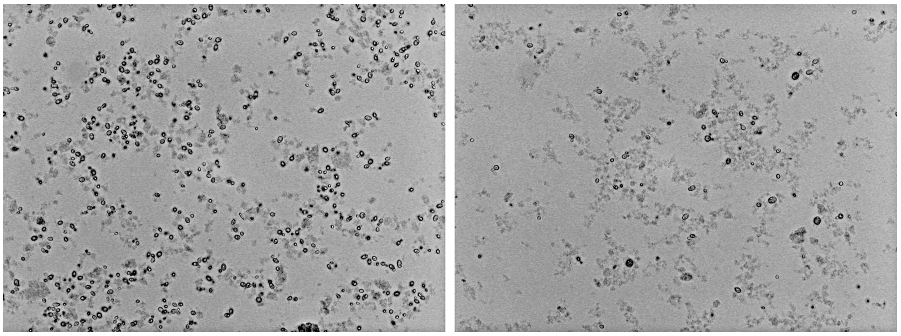


Figure 6: Light micrographs of *W. anomalus* J121 fermenter samples, showing a reduction in cell number. Samples were taken at 9 h (left), and 20 h.

The observed reduction in cells is indicative of cell lysis at or prior to approx. 20 h for strains *W. anomalus* J121 and *P. kudriavzevii* J550. For *W. anomalus* J121, oxygen consumption data suggests that lysis occurred around the 12-h mark. For *P. kudriavzevii* J550, it was not possible to draw any conclusions based on oxygen consumption as this was already decreasing due to a slowed rate of growth. No reduction in viable counts was observed in *B. adeninivorans* J564, but as noted above, the growth conditions were altered by the inconsistent aeration in this reactor.

The BMP assay was terminated after 40 days. Unfortunately, there was a problem with the calibration of the apparatus used for determining methane

production. Different AMPTS units were used for each of the triplicates, and after analyzing the data it became evident that the results deviated between the units, resulting in large variances. Therefore, comparisons between treatments were largely statistically insignificant. However, the trends that could be observed (Fig. 7) suggest that adding up to 50 % of supernatant from *W. anomalus* J121 or *B. adeninivorans* J564 fermentations did not influence methane potential negatively (i.e. compared with WS), and that addition of either *P. kudriavzevii* J550 supernatant or filtered control substrate may prove beneficial to methane production. Due to the large variances, however, the only statistically significant comparisons were WS+100% J550 compared to WS+100% J121 (+25 %, $p = 0.04$), and WS+100% control compared to WS+100% J121 (+30 %, $p = 0.02$). WS+100% J550 appeared to provide a 25 % increase over WS+100% J121, but this result did not reach statistical significance ($p = 0.06$). See Appendix B for all comparisons.

Due to equipment failure, the results for some of the samples with added control substrate had to be disregarded. Thus, the WS+50% control group is missing, and there are only two observations for WS+30% control and WS+100% control. Although repeating this trial would have been desirable, the length of the trial (40 days) unfortunately made this impossible within the time frame of this thesis work.

5 Scale-up experiments

5.1 Materials and methods

To establish reproducibility of results, obtain higher-quality growth curves, and produce enough biomass to allow for chemical analyses, bioreactor fermentations were repeated using four replicates. Fermentation parameters were the same as in the previous section (except for *B. adeninivorans* J564 which was cultured at 37 °C, the temperature at which it performed the best during screening). Fermentations were terminated when yeast growth was deemed to be slowing down after log phase growth, based on apparent oxygen consumption. For this trial, CFU and dry matter sampling was performed at intervals of 3 h (or more frequently during times of rapid growth).

After fermentation was complete, pellets and supernatants were collected as described in Section 4.1. Due to time constraints, methane potential of supernatants were not assessed. The *B. adeninivorans* J564 pellets were freeze-dried at an external lab and their weights determined there. Supernatants and filtered substrate were analyzed at an external lab (Agrilab AB, Uppsala,

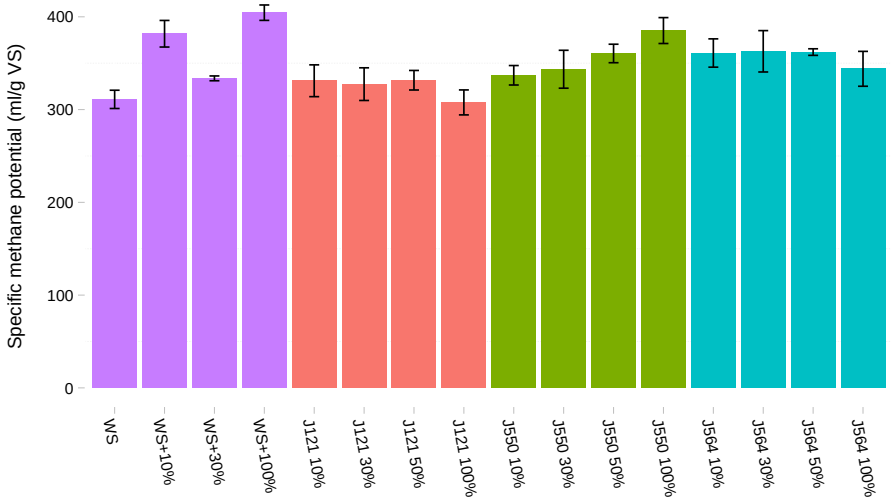


Figure 7: Specific methane potential for whole untreated substrate (WS) with variyng levels of post-fermentation supernatant added. 100 % refers to equal amounts of WS and supernatant in the vessels. Bars represent standard error ($n = 3$ for all samples, except WS+30% and WS+100% where $n = 2$).
Legend: WS, control with no addition; WS+X, WS with control supernatant (purple bars); J121 X%, WS with *W. anomalus* J121 supernatant (red bars); J550 X%, WS with *P. kudriavzevii* J550 supernatant (green bars); J564 X%, WS with *B. adeninivorans* J564 supernatant (blue bars).

Sweden).

5.2 Results

Growth curves from the scale-up fermentations are presented in Appendix C, Fig. C2 (CFU counts) and Fig. C3 (dry matter estimations). The fermentations were terminated when apparent growth was slowing down, and lasted for 12–15 h. No lysis was observed in this trial.

The dry matter yields from the scale-up fermentations ranged from 7.0–14.8 g per liter of substrate, and the protein contents ranged from 22.6–32.7 % while fat contents were similar. Micronutrient contents also varied between the yeast strains used, with Mg, K, and P exhibiting the largest relative differences. Crude energy was similar, but does not necessarily reflect the

amount of energy that can be utilized by the fish. The results of the chemical analyses of washed pellets are shown in Table 5.

Table 5: Nutritional characteristics of the washed pellets after fermentation of *W. anomalus* J121, *P. kudriavzevii* J550, and *B. adeninivorans* J564 in CSTR bioreactors. Values are expressed as means or as single result from pooled samples, and refers to contents in dry matter. Biomass concentration is given as g product per l of substrate. Standard errors are given where there was sufficient material to perform per-reactor analyses. Abbreviations: SE, standard error

	J121		J550		J564	
Parameter	**Value**	**SE**	**Value**	**SE**	**Value**	**SE**
Number of replicates[a]	3		4		3	
Biomass concentration (g/l)	7.03	0.04	7.36	0.17	14.8	0.59
Protein (%)	22.6	0.20	32.7	0.27	30.5	1.22
Ash (%)	45.8	0.24	42.9	0.42	37.6	1.69
Fat (%)	2.7	—	1.8	—	1.6	—
Crude energy (MJ/kg)	11.1	—	11.9	—	13.1	—
Ca (g/kg)	145.6	—	123.6	—	115.0	4.54
K (g/kg)	7.4	—	11.1	—	16.4	1.26
P (g/kg)	6.6	—	11.1	—	6.9	0.46
Mg (g/kg)	4.7	—	3.7	—	11.2	0.60
Na (g/kg)	83.3	—	85.3	—	76.7	1.47
S (g/kg)	3.0	—	3.6	—	4.3	0.23

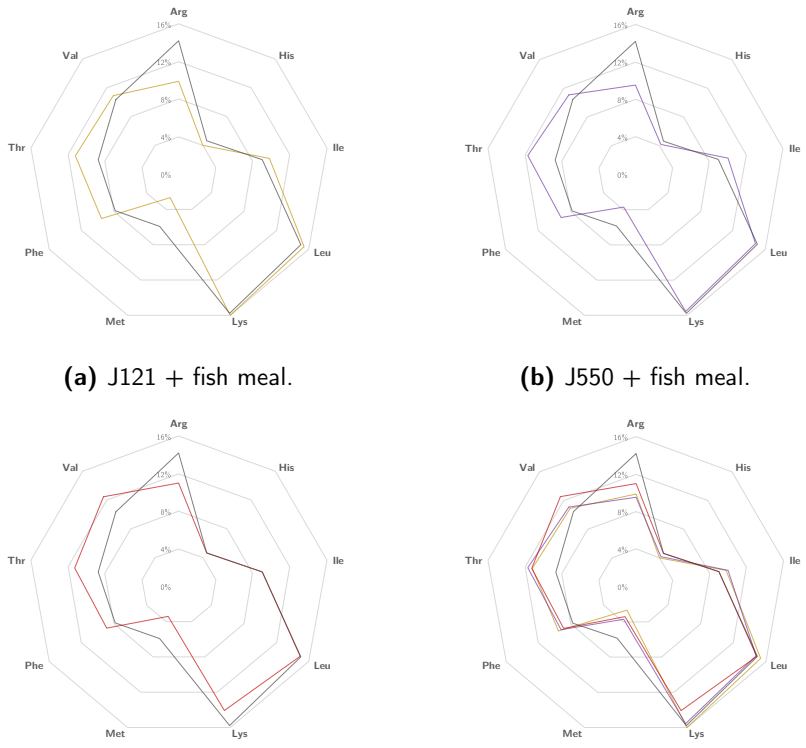
[a] All strains were inoculated in four reactors, but data for some replicates were discarded due to equipment failure.

Out of the three yeast strain, *B. adeninivorans* J564 was the best biomass producer, yielding approximately twice as much biomass as the other strains. Although the protein content of the *P. kudriavzevii* J550 pellets were somewhat higher, the greater productivity of *B. adeninivorans* J564 means that more protein can be produced in absolute terms. Regarding micronutrient content, *P. kudriavzevii* J550 pellets contained almost twice the amount of phosphorus compared to the other strains, while *B. adeninivorans* J564 contained high amounts of magnesium.

Amino acid composition was also dependent on yeast strain, although differences were smaller compared to those seen in total protein content. Comparing EAA composition of the yeast biomass to FM, pellets were generally deficient in methionine and arginine, but contained sufficient

lysine and abundant tyrosine, threonine and phenylalanine. Worth noting is that the profiles of *W. anomalus* J121 and *P. kudriavzevii* J550 are very similar, but *B. adeninivorans* J564 contained somewhat less lysine and more arginine than the other strains.

For a graphical representation of the relative EAA composition, see Fig. 8. The complete amino acid data is shown in Table D in Appendix D. Unfortunately, the method used for AA determination did not allow for quantification of tryptophan, which is essential for fish (Wilson and Halver, 1986).



(a) J121 + fish meal.

(b) J550 + fish meal.

(c) J564 + fish meal.

(d) All yeasts + fish meal.

Figure 8: Relative essential amino acid composition of yeast pellets. Legend: *W. anomalus* J121, yellow line; *P. kudriavzevii* J550, purple line; *B. adeninivorans* J564, red line; fish meal, grey line.

Chemical analysis of the filtered substrate (Table E in Appendix E) revealed a 3.7-fold reduction in TS, compared to the unfiltered whole substrate, due to the removal of particles. Total carbon was reduced by a factor of 4.5,

while nitrogen was only reduced by a factor of 2.3, ensuring an ample supply of the often limiting nutrient nitrogen to the yeast. Other micronutrients were not appreciably affected by filtration.

Yeast treatment altered the chemical composition of the substrate. All yeasts reduced the amount of C and N in the substrate, with the most productive strain *B. adeninivorans* J564 also consuming the highest amounts of both nutrients. See Table E in Appendix E for pre and post-fermentation chemical analysis data.

While yeast treatment generally reduced concentrations of micronutrients, concentrations of Na and P increased (the latter only for yeasts *P. kudriavzevii* J550 and *B. adeninivorans* J564). Excretion of Na^+ ions by yeast may occur concomitantly with K^+ ion uptake or simultaneously with excretion of organic acid anions (Conway et al., 1954).

Comparing Tables E and 5, reveals a result that is very hard to explain. It appears that *P. kudriavzevii* J550 has simultaneously sequestered P from the substrate, as evident by the high levels in the pellet, and at the same time increased the amount of P in the post-fermentation substrate. This suggests an error during analysis, perhaps by contamination of the sample or inappropriate sample preparation.

6 Discussion

To meet future demands for fish feed from a steadily growing aquaculture industry, sustainable sources of feed are needed as wild-caught fish production has already likely peaked. SCP has been shown to be potentially useful as a replacement for FM in fish feed, but its production depends on a steady supply of cheap substrate in order to be financially viable. In this thesis, it is demonstrated that yeast biomass can be produced on agricultural and household wastes, cheap and plentiful substrates which do not compete with human food interests. The results presented herein also suggest that a combination of yeast SCP production and biogas production may prove beneficial, although quite some work remains before a conclusive evaluation of such a venture is possible.

Determining proper growth conditions is of great importance for successfully implementing a large-scale production of microbial biomass. On the specific substrate/strain combinations evaluated here, pH exhibited the largest influence on growth. Furthermore, sterilization of the substrate proved to be crucial for successful yeast cultivation. Early experiments on this substrate, as well as previous work done using biogas substrate from another plant (unpublished data), showed little to no growth of the inocu-

lated yeast strains unless the material was first sterilized. This is likely due to competition by the more established indigenous microbial populations. Sterilization, or other means of reducing the likelihood or impact of contaminant growth, might also be a requirement for producing a consistent quality product, especially if a continuous production process is implemented.

Another important factor for successfully scaling up the process to industrial scale is the ability to cheaply and efficiently separate yeast biomass from the spent substrate. Although centrifugation, which was used in the experiments outlined here, could be a viable option, a more energy-efficient process based on passive techniques such as filtration could be beneficial from a cost-efficiency perspective (Fellows, 2009). The ease of separation of yeast cells could possibly be enhanced by the use of flocculating microorganisms such as *Lactobacillus vini*, which are able to aggregate with a number of yeast species (Tiukova et al., 2014; Matilda Olstorpe, personal communication, 2015).

During the screening phase of this work, certain outlier screening runs were identified. *Post hoc* analysis revealed a greater variance in weights recorded for empty Eppendorf tubes used for biomass determination in these runs, compared to the non-outlier experiments. The apparent cause for this discrepancy seems to be an imprecise analytical scale used for weighing resultant biomass, masses which were in the single-digit milligram range. It can also be assumed that this imprecision has affected the quality of measurements of dry pellet weights. If this method is to be employed in future work, using a well calibrated high-precision analytical scale should be considered a main priority.

Dry product yields from the scale-up experiments were 7.03 g after 12 h for *W. anomalus* J121, 7.36 g after 14 h for *P. kudriavzevii* J550, and 14.8 g after 15 h for *B. adeninivorans* J564, per liter of substrate. This corresponds to relative yields of 0.58, 0.53, and 0.99 g l^{-1} h^{-1}, respectively. Although the scope of this thesis does not allow for an exhaustive review of the literature on yeast-based SCP production, the results presented herein are presented alongside a few similar experiments (Table 6). Published data generally exhibits a large diversity, both in terms of substrate composition and realized yields. Within this very limited data set, *B. adeninivorans* J564 is very competitive, especially considering the potentially low cost of the substrate.

It is also apparent from these results that strain selection is an important aspect of SCP process development. Furthermore, the importance of scale-up experiments is highlighted by the fact that these results were not fully reflected during the screening runs. It is likely that very oxygen-demanding strains are unable to perform optimally when cultured in aerated 96-well

plates.

Table 6: Biomass yield, protein content, and productivity of the yeasts evaluated compared to previously published data from similar experiments. Abbreviations: conc, concentration

Organism	Conc. (g/l)	Protein (%)	Productivity (g/l·h)
W. anomalus J121[a]	7.0	22.6	0.58
P. kudriavzevii J550[a]	7.4	32.7	0.53
B. adeninivorans J564[a]	14.8	30.5	0.99
Deb. hansenii[b]	—	31.8	0.47
Yeast co-culture[c]	6.1	43.4	0.20
Cr. aureus[d]	10.1	53.0	0.18
K. marxianus[e]	50	—	approx. 1.4

[a] Evaluated in the present study.

[b] Cultured on hemicellulosic hydrolysate with nutrient supplementation (Duarte et al., 2008).

[c] *K. marxianus* and *C. krusei* cultured on cheese whey (Yadav et al., 2014).

[d] Cultured on Jerusalem artichoke extract and soybean hydrolysate (Gao et al., 2007).

[e] Cultured on deproteinized whey concentrate with nitrogen supplementation (Schultz et al., 2006).

Judging by the growth curves (Figs. C2 and C3), productivity could likely be increased by terminating the fermentations earlier. It is worth noting that biomass yields and viable counts do not always correlate perfectly. For *W. anomalus* J121 the biomass yield and viable count plots line up well. For the other two yeast strains, however, there seems to exist a phase where biomass increases exponentially separately from the log phase seen in the CFU plots. Additionally, protein yield would likely be higher if the fermentation is terminated at the optimum fermentation stage. Data from an earlier trial with *W. anomalus* J121 suggest a decrease in protein content from 52.1 % after 24 h to 35.5 % after 48 h of fermentation (Simon Isaksson, personal communication, 2015). Production can be expected to increase even further if the process is implemented as, and optimized for, a continuous process Goldberg (1985).

Yeast biomass produced during the scale-up fermentations looks well-suited as a fish feed ingredient. Protein contents are high, and EAA composition is similar to bakers yeast (*Saccharomyces cerevisiae*) which is already used as an ingredient in fish feed formulations (Tacon et al., 2009). Furthermore,

levels of tryptophan (an EAA that was not evaluated here) are generally higher in baker's yeast than in fish meal, suggesting that other yeast biomass also contain sufficient amounts of this EAA.

The results of the BMP assay are hard to interpret due to the equipment issues encountered during these experiments. No yeast treated substrate was able to significantly outperform WS, although the best-performing treatment (WS+100% J550) came close at $p = 0.06$. However, there are apparent trends in the data which suggest that the experiment is worth repeating on well-calibrated equipment. Using only the highest-performing strains and addition levels would also allow use of more replicates, ensuring higher statistical power. It is worth reiterating that the preparatory fermenter runs for the BMP assay were not conducted without issues.

If indeed the trends observed in the BMP assay are indicative of a true positive effect of yeast treatment on methane production, there are several possibilities as to why this would happen. As filtered substrate contains more easily available energy as the particles have been filtered out. This in itself would increase methane output (measured in ml g^{-1} VS^{-1}), and was controlled for in the WS+X% control samples. If the yeast would consume all of the energy available in the filtered substrate, final BMP results would be lower for the yeast treated samples than the WS control, with the WS+100% yeast level being the lowest since more VS, in absolute terms, would be consumed. However, at least for *P. kudriavzevii* J550, there was an apparent increase in methane output correlating with larger additions of yeast treated supernatant.

Yeasts, being eukaryotic organisms, can be assumed to be less closely related to the organisms making up an AD culture. As such, enzymes present in yeasts may not be present in an AD culture, or may be different enough that digestion is more complete after yeast enzymatic degradation. Thus, yeast may consume energy-containing compounds which are not utilized by the AD flora. In addition, these still-active enzymes may remain in the supernatant after biomass harvesting, and thus be introduced into the digester. In the digester, they may aid in the breakdown of organic matter and thereby facilitate the AD process.

Furthermore, yeasts may influence the chemical makeup of the substrate in more subtle ways, affecting the AD breakdown process either directly, by facilitating AD digestion; or indirectly, by altering the dynamics of the complex AD microflora. Such alterations could include micronutrient content (or the chemical forms in which these nutrients exists) or the alteration of levels of inhibitory compounds present in the substrate.

A puzzling observation during the first fermenter experiment was the

apparent cell lysis observed (Section 4.2). The reason for this is not known, and lysis was not observed in subsequent bioreactor fermentations. As the fermentations were carried out according to the same protocol, methodological error can be ruled out. Contamination by a competing microorganism could be a possible explanation, but no microbial growth was observed in the uninoculated control. Because chloramphenicol-containing media was used for the CFU counts, a bacterial contamination would not be detected on the plates, but no bacteria were seen under the microscope. The same batch of fermentation substrate was used for all reactor fermentations, ruling out the possibility of toxic substances being present in the medium.

Finally, although this thesis provides a good starting point for scaling up the process described herein, additional research is warranted for attaining a more optimized process. Higher-resolution growth curves should be generated, and additional samples should be taken at each time point and assayed for both biomass and CP, to establish the proper fermentation duration. Furthermore, fine-grained temperature screening should be performed, and pH optima should be reevaluated after a production strain has been chosen. Finally, if cell lysis is observed during these experiments, effort should be made to understand its cause.

7 Conclusions

In conclusion, the data presented in this thesis suggest that combining biogas and SCP production is possible, and that the combination might be beneficial to biogas production by providing a valuable by-product, while at the same time not reducing biogas output. Large-scale implementation of such a system could lead to environmental benefits, as SCP-based feedstuffs could reduce the strain on wild fish stocks by substituting fish meal derived from wild-caught fish.

The methods used in this thesis allow time-efficient screening of yeast biomass production under multiple growth conditions, as well as evaluation of the effects of yeast treatment on biogas production. As the growth parameters and biogas output effects likely vary between different biogas substrates, the methods presented here could be used for estimating the suitability of a specific biogas substrate for SCP production. Analyses of the final products show a large diversity in product yields and protein contents, further demonstrating the need for screening for optimal production strains.

References

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., Kalyuzhnyi, S., Jenicek, P. and van Lier, J. B. (2009). Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays, *Water Science & Technology* **59**(5): 927–934.

Angelidaki, I., Karakashev, D., Batstone, D. J., Plugge, C. M. and Stams, A. J. M. (2011). Biomethanation and Its Potential, *Methods in Methane Metabolism, Part A*, Elsevier, Cambridge, pp. 327–351.

Braude, R. and Rhodes, D. N. (1977). Pruteen, a new source of protein for growing pigs. II. Feeding trial: Growth rate, feed utilization and carcass and meat quality, *Livestock Production Science* **4**(1): 91–100.

Coelho, M., Amaral, P. and Belo, I. (2010). *Yarrowia lipolytica*: an industrial workhorse, *in* A. Mendez-Vilas (ed.), *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, Badajoz, Spain.

Conway, E. J., Ryan, H. and Carton, E. (1954). Active transport of sodium ions from the yeast cell, *The Biochemical journal* **58**(1): 158–167.

Duarte, L. C., Carvalheiro, F., Lopes, S., Neves, I. and Gírio, F. M. (2008). Yeast biomass production in brewery's spent grains hemicellulosic hydrolyzate, *Applied biochemistry and biotechnology* **148**(1-3): 119–129.

Eliasson, T. (2015). *Dairy waste – Feed for fish?*, Bachelor's thesis, SLU, Uppsala.
URL: *http://stud.epsilon.slu.se/8356/*

Esposito, G., Frunzo, L., Liotta, F., Panico, A. and Pirozzi, F. (2012). Bio-Methane Potential Tests To Measure The Biogas Production From The Digestion and Co-Digestion of Complex Organic Substrates, *The Open Environmental Engineering Journal* **5**(1): 1–8.

FAO (2011). *Demand and supply of feed ingredients for farmed fish and crustaceans*, Trends and prospects, FAO, Rome.

FAO (2014). *The State of World Fisheries and Aquaculture*, Opportunities and challenges, FAO, Rome.

FAO (2016). *FAOSTAT*, Food and Agriculture Organization, Rome.
URL: *http://faostat3.fao.org/*

FEAP Secretariat (2015). European Aquaculture Production Report 2005–2014, *Technical report*, FEAP Secretariat, Liege.

Fellows, P. J. (2009). *Food Processing Technology*, Principles and Practice, third edition edn, Woodhead Publishing, Cambridge.

Gao, L., Chi, Z., Sheng, J., Ni, X. and Wang, L. (2007). Single-cell protein production from Jerusalem artichoke extract by a recently isolated marine yeast *Cryptococcus aureus* G7a and its nutritive analysis, *Applied Microbiology and Biotechnology* **77**(4): 825–832.

Goldberg, I. (1985). *Single Cell Protein*, Vol. 1, Springer Berlin Heidelberg, Berlin, Heidelberg.

Matelbs, R. I. and Tannenbaum, S. E. (1968). Single-Cell protein, *Economic Botany* **22**(1): 42–50.

Middelhoven, W. J., de Jong, I. M. and de Winter, M. (1991). *Arxula adeninivorans*, a yeast assimilating many nitrogenous and aromatic compounds, *Antonie van Leeuwenhoek* **59**(2): 129–137.

Naylor, R. L., Hardy, R. W., Bureau, D. P., Chiu, A., Elliott, M., Farrell, A. P., Forster, I., Gatlin, D. M., Goldburg, R. J., Hua, K. and Nichols, P. D. (2009). Feeding aquaculture in an era of finite resources, *Proceedings of the National Academy of Sciences of the United States of America* **106**(36): 15103–15110.

Nordic Committee on Food Analysis (2003). *NMKL 6. Nitrogen. Determination in foods and feeds according to Kjeldahl*, 4 edn, NMKL, Oslo.

Passoth, V., Fredlund, E., Druvefors, U. A. and Schnürer, J. (2006). Biotechnology, physiology and genetics of the yeast *Pichia anomala*, *FEMS yeast research* **6**(1): 3–13.

R Core Team (2015). *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria.
URL: *https://www.R-project.org*

Radecka, D., Mukherjee, V., Mateo, R. Q., Stojiljkovic, M., Foulquié-Moreno, M. R. and Thevelein, J. M. (2015). Looking beyond *Saccharomyces*: the potential of non-conventional yeast species for desirable traits in bioethanol fermentation, *FEMS yeast research* **15**(6): fov053.

Rumsey, G. L., Winfree, R. A. and Hughes, S. G. (1992). Nutritional value of dietary nucleic acids and purine bases to rainbow trout (*Oncorhynchus mykiss*), *Aquaculture* **108**(1-2): 97–110.

Schnürer, J. and Jonsson, A. (2010). *Pichia anomala* J121: a 30-year overnight near success biopreservation story, *Antonie van Leeuwenhoek* **99**(1): 5–12.

Schultz, N., Chang, L., Hauck, A., Reuss, M. and Syldatk, C. (2006). Microbial production of single-cell protein from deproteinized whey concentrates, *Applied Microbiology and Biotechnology* **69**(5): 515–520.

Tacon, A. G. J. and Metian, M. (2009). Fishing for feed or fishing for food: increasing global competition for small pelagic forage fish, *Ambio* **38**(6): 294–302.

Tacon, A. G. J., Metian, M. and Hasan, M. R. (2009). Feed Ingredients and Fertilizers for Farmed Aquatic Animals, *Technical Report 540*, Rome.

The Commission of the European Communities (1998). COMMISSION DIRECTIVE 98/64/EC of 3 September 1998, establishing Community methods of analysis for the determination of amino- acids, crude oils and fats, and olaquindox in feedingstuffs and amending Directive 71/393/EEC, *Official Journal of the European Communities* **L 257/14**.

Tiukova, I., Eberhard, T. and Passoth, V. (2014). Interaction of *Lactobacillus vini* with the ethanol-producing yeasts *Dekkera bruxellensis* and *Saccharomyces cerevisiae*, *Biotechnology and Applied Biochemistry* **61**(1): 40–44.

Vidaković, A. (2015). *Fungal and Mussel Protein Sources in Fish Feed*, PhD thesis, Swedish University of Agricultural Sciences, Uppsala.
URL: *http://pub.epsilon.slu.se/12523/*

Waites, M. J., Morgan, N. L., Rockey, J. S. and Higton, G. (2009). *Industrial Microbiology*, An Introduction, John Wiley & Sons, Oxford.

Weiland, P. (2009). Biogas production: current state and perspectives, *Applied Microbiology and Biotechnology* **85**(4): 849–860.

Wiebe, M. G. (2004). Quorn™ Myco-protein – Overview of a successful fungal product, *Mycologist* **18**(01): 17–20.

Wilson, R. P. and Halver, J. E. (1986). Protein and amino acid requirements of fishes, *Annual Review of Nutrition* **6**(1): 225–244.

Yadav, J. S. S., Bezawada, J., Ajila, C. M., Yan, S., Tyagi, R. D. and Surampalli, R. Y. (2014). Mixed culture of *Kluyveromyces marxianus* and *Candida krusei* for single-cell protein production and organic load removal from whey, *Bioresource technology* **164**: 119–127.

Acknowledgements

During the writing of this thesis, the support and suggestions I've received from my supervisors and from my colleagues at the Department of Microbiology has been immeasurably valuable. Su-lin, thank you for your excellent feedback on my drafts, and for being a constant source of inspiration and encouragement during the long hours spent in the lab. Simon, thank you for helping me out with the biogas assays. Our discussions on methodology were always very productive, and likely spared me from making a few mistakes along the way. Thomas and Albina, thank you for all your help with running the fermenters. Finally, I'm grateful to Matilda for giving me the opportunity to perform this research, a process which has been both interesting and rewarding.

Support for this work was provided by Karlskoga Energi & Miljö and the NJ faculty at the Swedish University of Agricultural Sciences.

Appendices

A Screening results

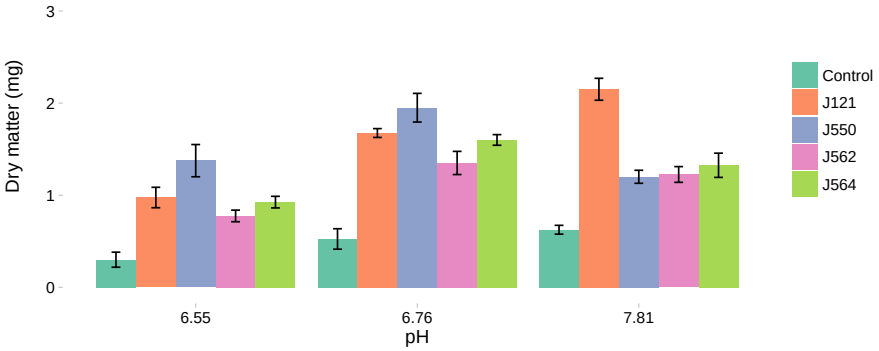


Figure A1: Screening results for 30 °C, comparing strains *W. anomalus* J121, *P. kudriavzevii* J550, and *B. adeninivorans* J562 and J564. Bars represent standard error ($n = 4$).

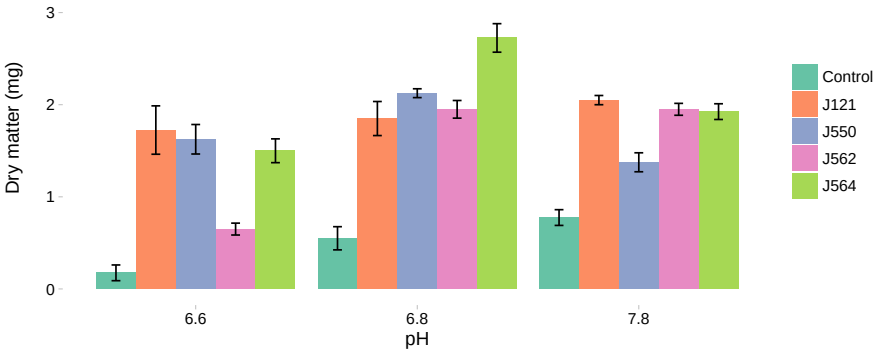


Figure A2: Screening results for 25 °C, comparing strains *W. anomalus* J121, *P. kudriavzevii* J550, and *B. adeninivorans* J562 and J564. Bars represent standard error ($n = 4$).

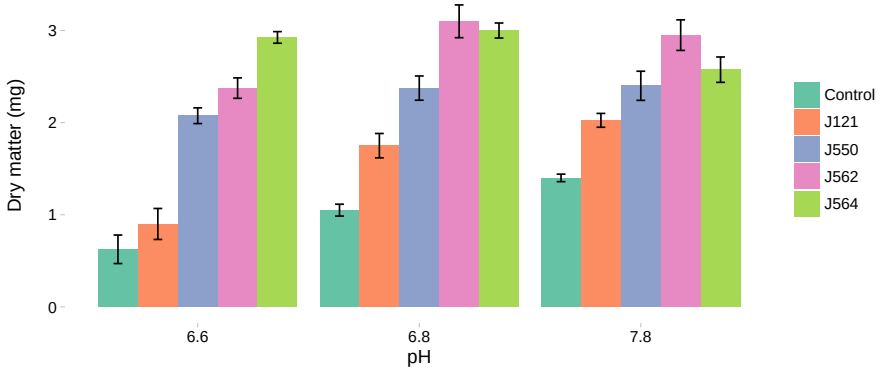


Figure A3: Screening results for 37 °C, comparing strains *W. anomalus* J121, *P. kudriavzevii* J550, and *B. adeninivorans* J562 and J564. Bars represent standard error ($n = 4$).

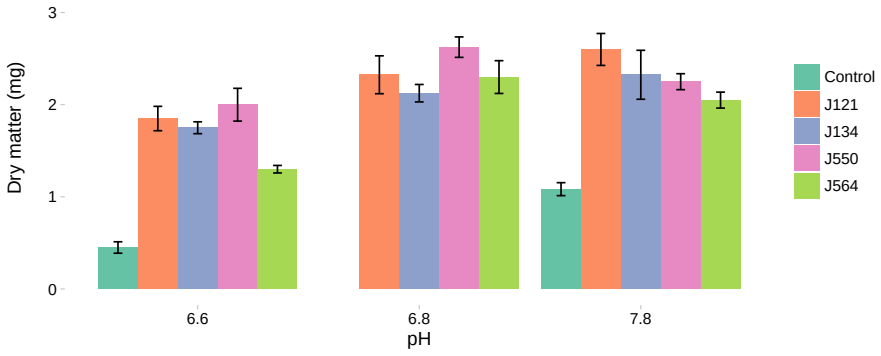


Figure A4: Screening results for 30 °C, comparing strains *W. anomalus* J121, *P. kudriavzevii* J550, *Y. lipolytica* J134 and *B. adeninivorans* J564. Bars represent standard error ($n = 4$).

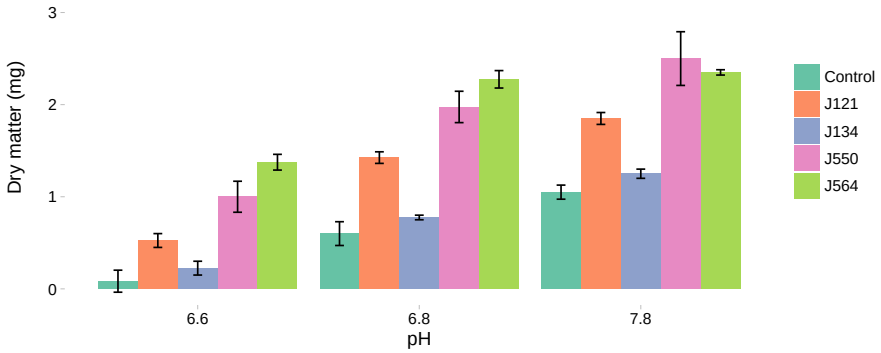


Figure A5: Screening results for 30 °C, comparing strains *W. anomalus* J121, *P. kudriavzevii* J550, *Y. lipolytica* J134 and *B. adeninivorans* J564. Bars represent standard error ($n = 4$).

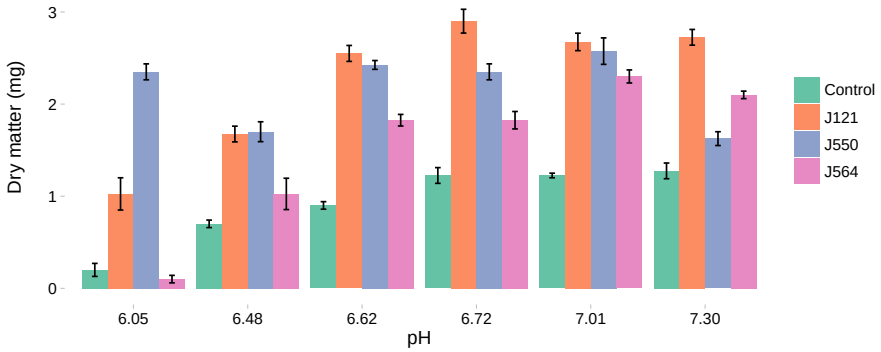


Figure A6: Screening results for determining optimum pH. comparing strains *W. anomalus* J121, *P. kudriavzevii* J550, and *B. adeninivorans* J564. Bars represent standard error ($n = 4$).

B Tukey's HSD results for BMP assays

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = lm(value ~ Sample, bb.melt))

$Sample
                              diff         lwr        upr     p adj
J121 10%-Background  331.0788780  255.060083 407.097673 0.0000000
J121 100%-Background 307.7347041  231.715909 383.753499 0.0000000
J121 30%-Background  327.4345146  251.415719 403.453310 0.0000000
J121 50%-Background  331.6509882  255.632193 407.669784 0.0000000
J550 10%-Background  336.9987019  260.979906 413.017497 0.0000000
J550 100%-Background 385.1959481  309.177153 461.214743 0.0000000
J550 30%-Background  343.4968508  267.478055 419.515646 0.0000000
J550 50%-Background  360.5020159  284.483221 436.520811 0.0000000
J564 10%-Background  360.9943613  284.975566 437.013157 0.0000000
J564 100%-Background 343.9552791  267.936484 419.974074 0.0000000
J564 30%-Background  362.8367914  286.817996 438.855587 0.0000000
J564 50%-Background  362.0006476  285.981852 438.019443 0.0000000
WS-Background        311.0236684  235.004873 387.042464 0.0000000
WS+10%-Background    381.7618554  305.743060 457.780651 0.0000000
WS+100%-Background   404.4635162  319.471919 489.455113 0.0000000
WS+30%-Background    333.6740168  248.682420 418.665614 0.0000000
J121 100%-J121 10%   -23.3441739  -99.362969  52.674621 0.9986493
J121 30%-J121 10%     -3.6443634  -79.663159  72.374432 1.0000000
J121 50%-J121 10%      0.5721102  -75.446685  76.590906 1.0000000
J550 10%-J121 10%      5.9198238  -70.098972  81.938619 1.0000000
J550 100%-J121 10%    54.1170701  -21.901725 130.135865 0.4155072
J550 30%-J121 10%     12.4179728  -63.600823  88.436768 0.9999997
J550 50%-J121 10%     29.4231379  -46.595658 105.441933 0.9852879
J564 10%-J121 10%     29.9154833  -46.103312 105.934279 0.9828600
J564 100%-J121 10%    12.8764011  -63.142394  88.895196 0.9999994
J564 30%-J121 10%     31.7579133  -44.260882 107.776709 0.9709482
J564 50%-J121 10%     30.9217696  -45.097026 106.940565 0.9769443
WS-J121 10%          -20.0552096  -96.074005  55.963586 0.9997754
WS+10%-J121 10%       50.6829774  -25.335818 126.701773 0.5225356
WS+100%-J121 10%      73.3846382  -11.606959 158.376235 0.1537256
WS+30%-J121 10%        2.5951388  -82.396458  87.586736 1.0000000
J121 30%-J121 100%    19.6998105  -56.318985  95.718606 0.9998199
```

J121 50%-J121 100%	23.9162841	-52.102511	99.935079	0.9982294
J550 10%-J121 100%	29.2639977	-46.754798	105.282793	0.9860123
J550 100%-J121 100%	77.4612440	1.442449	153.480039	0.0422400
J550 30%-J121 100%	35.7621467	-40.256649	111.780942	0.9256877
J550 50%-J121 100%	52.7673118	-23.251484	128.786107	0.4565514
J564 10%-J121 100%	53.2596572	-22.759138	129.278453	0.4413976
J564 100%-J121 100%	36.2205750	-39.798220	112.239370	0.9185445
J564 30%-J121 100%	55.1020873	-20.916708	131.120883	0.3866430
J564 50%-J121 100%	54.2659435	-21.752852	130.284739	0.4110818
WS-J121 100%	3.2889643	-72.729831	79.307760	1.0000000
WS+10%-J121 100%	74.0271513	-1.991644	150.045947	0.0628282
WS+100%-J121 100%	96.7288121	11.737215	181.720409	0.0139039
WS+30%-J121 100%	25.9393127	-59.052284	110.930910	0.9987401
J121 50%-J121 30%	4.2164736	-71.802322	80.235269	1.0000000
J550 10%-J121 30%	9.5641872	-66.454608	85.582983	1.0000000
J550 100%-J121 30%	57.7614335	-18.257362	133.780229	0.3141896
J550 30%-J121 30%	16.0623362	-59.956459	92.081132	0.9999873
J550 50%-J121 30%	33.0675012	-42.951294	109.086297	0.9593360
J564 10%-J121 30%	33.5598467	-42.458949	109.578642	0.9542089
J564 100%-J121 30%	16.5207645	-59.498031	92.539560	0.9999815
J564 30%-J121 30%	35.4022767	-40.616519	111.421072	0.9309976
J564 50%-J121 30%	34.5661329	-41.452662	110.584928	0.9423413
WS-J121 30%	-16.4108462	-92.429642	59.607949	0.9999830
WS+10%-J121 30%	54.3273408	-21.691455	130.346136	0.4092630
WS+100%-J121 30%	77.0290016	-7.962595	162.020599	0.1101348
WS+30%-J121 30%	6.2395022	-78.752095	91.231099	1.0000000
J550 10%-J121 50%	5.3477136	-70.671082	81.366509	1.0000000
J550 100%-J121 50%	53.5449599	-22.473835	129.563755	0.4327090
J550 30%-J121 50%	11.8458626	-64.172933	87.864658	0.9999998
J550 50%-J121 50%	28.8510277	-47.167768	104.869823	0.9877623
J564 10%-J121 50%	29.3433731	-46.675422	105.362168	0.9856546
J564 100%-J121 50%	12.3042909	-63.714504	88.323086	0.9999997
J564 30%-J121 50%	31.1858032	-44.832992	107.204599	0.9751625
J564 50%-J121 50%	30.3496594	-45.669136	106.368455	0.9804715
WS-J121 50%	-20.6273198	-96.646115	55.391476	0.9996831
WS+10%-J121 50%	50.1108672	-25.907928	126.129663	0.5410213
WS+100%-J121 50%	72.8125280	-12.179069	157.804125	0.1616916
WS+30%-J121 50%	2.0230286	-82.968568	87.014626	1.0000000
J550 100%-J550 10%	48.1972462	-27.821549	124.216042	0.6032870
J550 30%-J550 10%	6.4981490	-69.520646	82.516944	1.0000000

J550 50%-J550 10%	23.5033140	-52.515481	99.522109	0.9985421
J564 10%-J550 10%	23.9956595	-52.023136	100.014455	0.9981632
J564 100%-J550 10%	6.9565773	-69.062218	82.975373	1.0000000
J564 30%-J550 10%	25.8380895	-50.180706	101.856885	0.9959270
J564 50%-J550 10%	25.0019457	-51.016850	101.020741	0.9971258
WS-J550 10%	-25.9750334	-101.993829	50.043762	0.9956959
WS+10%-J550 10%	44.7631536	-31.255642	120.781949	0.7125294
WS+100%-J550 10%	67.4648144	-17.526783	152.456411	0.2524849
WS+30%-J550 10%	-3.3246850	-88.316282	81.666912	1.0000000
J550 30%-J550 100%	-41.6990973	-117.717893	34.319698	0.8006724
J550 50%-J550 100%	-24.6939322	-100.712728	51.324863	0.9974855
J564 10%-J550 100%	-24.2015868	-100.220382	51.817209	0.9979817
J564 100%-J550 100%	-41.2406690	-117.259464	34.778126	0.8126905
J564 30%-J550 100%	-22.3591567	-98.377952	53.659639	0.9991750
J564 50%-J550 100%	-23.1953005	-99.214096	52.823495	0.9987435
WS-J550 100%	-74.1722797	-150.191075	1.846516	0.0618026
WS+10%-J550 100%	-3.4340927	-79.452888	72.584703	1.0000000
WS+100%-J550 100%	19.2675681	-65.724029	104.259165	0.9999675
WS+30%-J550 100%	-51.5219313	-136.513528	33.469666	0.6713831
J550 50%-J550 30%	17.0051651	-59.013630	93.023960	0.9999728
J564 10%-J550 30%	17.4975105	-58.521285	93.516306	0.9999604
J564 100%-J550 30%	0.4584283	-75.560367	76.477224	1.0000000
J564 30%-J550 30%	19.3399406	-56.678855	95.358736	0.9998570
J564 50%-J550 30%	18.5037968	-57.514999	94.522592	0.9999183
WS-J550 30%	-32.4731824	-108.491978	43.545613	0.9649594
WS+10%-J550 30%	38.2650046	-37.753791	114.283800	0.8814587
WS+100%-J550 30%	60.9666654	-24.024932	145.958262	0.4032792
WS+30%-J550 30%	-9.8228340	-94.814431	75.168763	1.0000000
J564 10%-J550 50%	0.4923454	-75.526450	76.511141	1.0000000
J564 100%-J550 50%	-16.5467368	-92.565532	59.472059	0.9999811
J564 30%-J550 50%	2.3347755	-73.684020	78.353571	1.0000000
J564 50%-J550 50%	1.4986317	-74.520164	77.517427	1.0000000
WS-J550 50%	-49.4783475	-125.497143	26.540448	0.5615638
WS+10%-J550 50%	21.2598395	-54.758956	97.278635	0.9995442
WS+100%-J550 50%	43.9615003	-41.030097	128.953097	0.8590013
WS+30%-J550 50%	-26.8279991	-111.819596	58.163598	0.9981632
J564 100%-J564 10%	-17.0390822	-93.057878	58.979713	0.9999721
J564 30%-J564 10%	1.8424301	-74.176365	77.861225	1.0000000
J564 50%-J564 10%	1.0062863	-75.012509	77.025082	1.0000000
WS-J564 10%	-49.9706929	-125.989488	26.048102	0.5455660

WS+10%-J564 10%	20.7674941	-55.251301	96.786289	0.9996560
WS+100%-J564 10%	43.4691549	-41.522442	128.460752	0.8686406
WS+30%-J564 10%	-27.3203445	-112.311941	57.671252	0.9977568
J564 30%-J564 100%	18.8815123	-57.137283	94.900308	0.9998943
J564 50%-J564 100%	18.0453685	-57.973427	94.064164	0.9999408
WS-J564 100%	-32.9316107	-108.950406	43.087185	0.9606755
WS+10%-J564 100%	37.8065763	-38.212219	113.825372	0.8905176
WS+100%-J564 100%	60.5082371	-24.483360	145.499834	0.4154136
WS+30%-J564 100%	-10.2812623	-95.272859	74.710335	1.0000000
J564 50%-J564 30%	-0.8361438	-76.854939	75.182652	1.0000000
WS-J564 30%	-51.8131229	-127.831918	24.205672	0.4864354
WS+10%-J564 30%	18.9250640	-57.093731	94.943859	0.9998912
WS+100%-J564 30%	41.6267248	-43.364872	126.618322	0.9012653
WS+30%-J564 30%	-29.1627746	-114.154372	55.828822	0.9955045
WS-J564 50%	-50.9769792	-126.995775	25.041816	0.5130841
WS+10%-J564 50%	19.7612078	-56.257588	95.780003	0.9998129
WS+100%-J564 50%	42.4628686	-42.528728	127.454466	0.8871419
WS+30%-J564 50%	-28.3266308	-113.318228	56.664966	0.9966880
WS+10%-WS	70.7381870	-5.280608	146.756982	0.0904720
WS+100%-WS	93.4398478	8.448251	178.431445	0.0201256
WS+30%-WS	22.6503484	-62.341249	107.641945	0.9997456
WS+100%-WS+10%	22.7016608	-62.289936	107.693258	0.9997385
WS+30%-WS+10%	-48.0878386	-133.079436	36.903758	0.7644604
WS+30%-WS+100%	-70.7894994	-163.893129	22.314130	0.3132186

C Fermenter growth curves

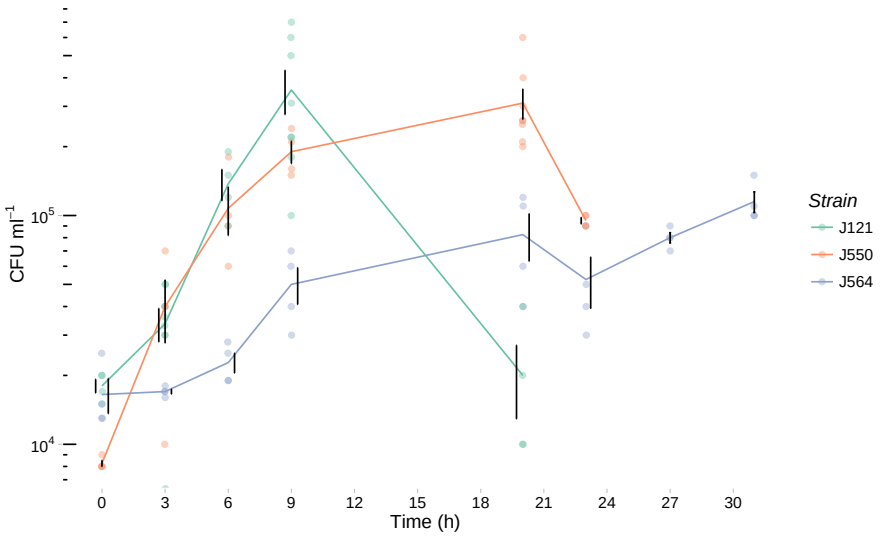
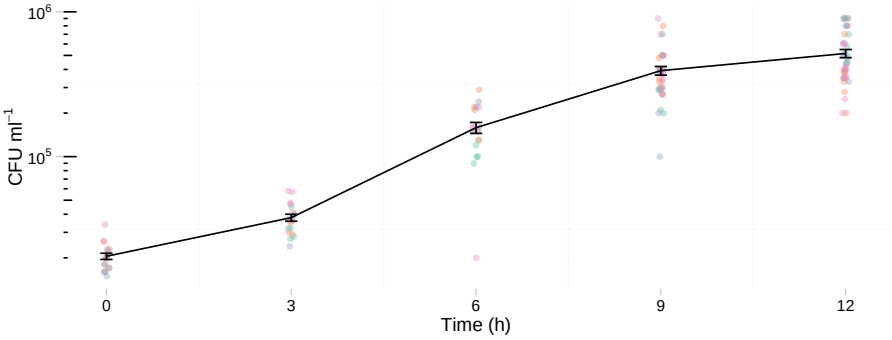
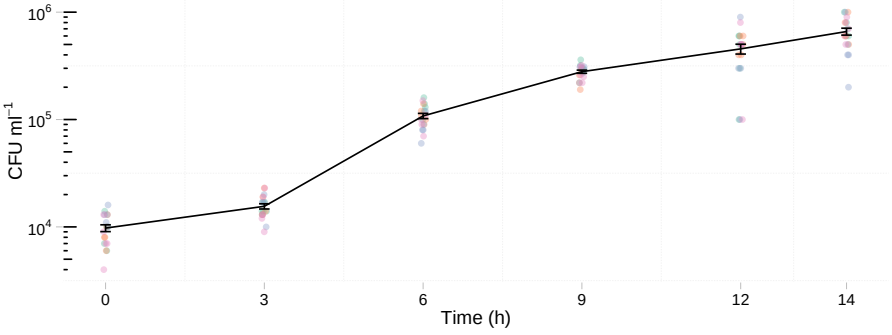


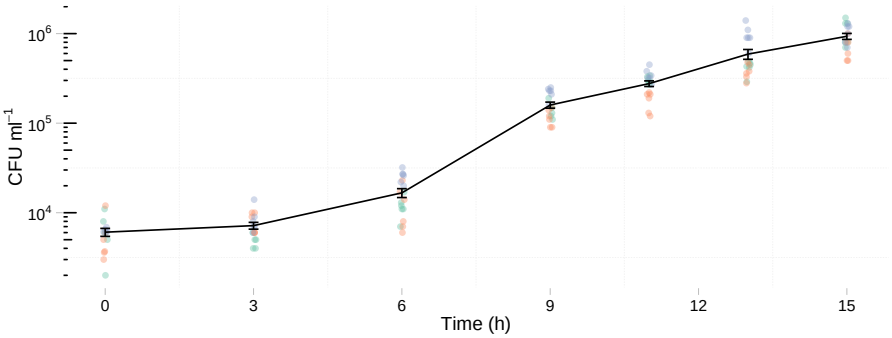
Figure C1: CFU counts for cultivation of yeast strains on filtered biogas substrate, during substrate preparation for the BMP assay. Tested strains were *W. anomalus* J121, *P. kudriavzevii* J550, and *B. adeninivorans* J564. Dots represent individual measurements, color coded by reactor, and are slightly jittered in x-direction to increase legibility. Bars represent standard error (generally $n = 4$).



(a) *W. anomalus* J121.

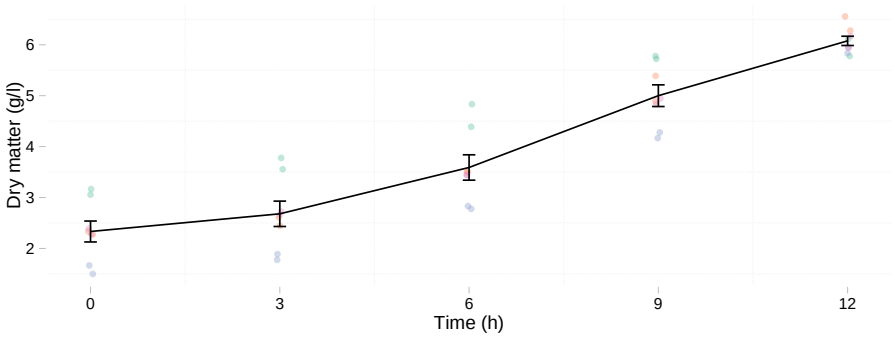


(b) *P. kudriavzevii* J550.

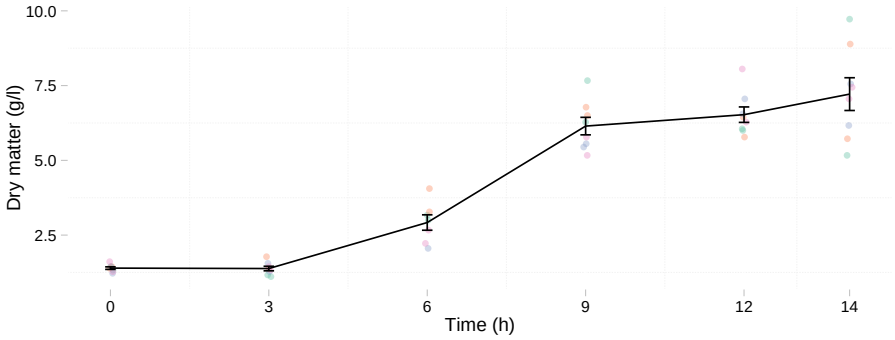


(c) *B. adeninivorans* J564.

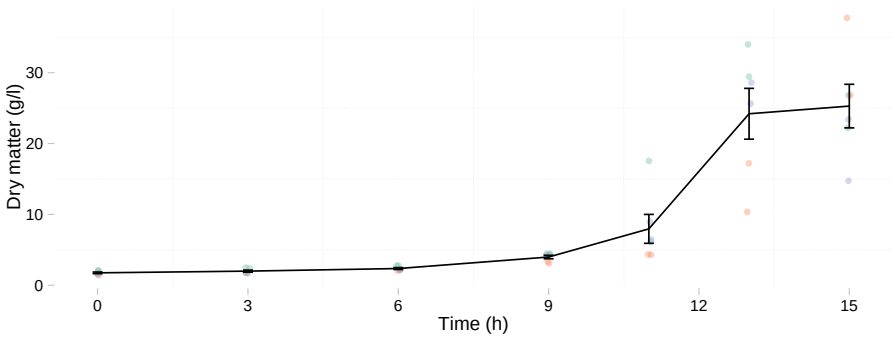
Figure C2: CFU counts from the scale-up fermentations. Dots represent individual samples, color-coded by reactor, and are slightly jittered in x-direction to increase legibility. Bars represent standard error.



(a) *W. anomalus* J121.



(b) *P. kudriavzevii* J550.



(c) *B. adeninivorans* J564.

Figure C3: Dry matter estimates from the scale-up fermentations. Dots represent individual samples, color-coded by reactor, and are slightly jittered in x-direction to increase legibility. Bars represent standard error.

D Amino acid composition

Table D: Amino acid composition of the final washed pellets. Values are given as absolute contents of dry matter, and as proportions of all analyzed amino acids. Abbreviations: Rel, relative composition

	J121		J550		J564	
	g/100 g	Rel.	g/100 g	Rel.	g/100 g	Rel.
Alanine	1.55	8.8 %	1.71	6.4 %	1.93	7.7 %
Arginine	0.87	5.0 %	1.35	5.0 %	1.36	5.4 %
Aspartic acid	1.88	10.7 %	3.32	12.4 %	2.42	9.7 %
Cysteine	0.157	0.9 %	0.274	1.0 %	0.275	1.1 %
Glutamic acid	2.63	15.0 %	3.73	13.9 %	4.08	16.4 %
Glycine	0.84	4.8 %	1.30	4.9 %	1.28	5.1 %
Histidine	0.351	2.0 %	0.594	2.2 %	0.572	2.3 %
Hydroxyproline	<0.05	—	<0.05	—	<0.05	—
Isoleucine	0.87	4.9 %	1.42	5.3 %	1.10	4.4 %
Leucine	1.36	7.7 %	2.11	7.9 %	1.84	7.4 %
Lysine	1.41	8.0 %	2.22	8.3 %	1.74	7.0 %
Methionine	0.242	1.4 %	0.529	2.0 %	0.417	1.7 %
Ornithine	<0.01	—	<0.01	—	<0.01	—
Phenylalanine	0.84	4.8 %	1.31	4.9 %	1.09	4.4 %
Proline	0.75	4.3 %	1.06	3.9 %	1.44	5.8 %
Serine	1.06	6.1 %	1.49	5.6 %	1.44	5.8 %
Threonine	0.99	5.6 %	1.66	6.2 %	1.39	5.6 %
Tyrosine	0.76	4.4 %	1.15	4.3 %	1.00	4.0 %
Valine	0.96	5.5 %	1.58	5.9 %	1.55	6.2 %

E Substrate composition

Table E: Chemical characterization of the biogas substrate during different stages of processing. *Unfiltered* refers to the substrate without any treatment. *Filtered* substrate refers to the substrate as it was used for the fermentation trials. Columns *J121*, *J550*, and *J564* refers to the substrate post-fermentation, after removal of biomass, from the three different yeasts *W. anomalus* J121, *P. kudriavzevii* J550, and *B. adeninivorans* J564. Abbreviations: TS, total solids; VS, volatile solids

	Unfiltered	**Filtered**	**J121**	**J550**	**J564**
TS (%)	7.8	2.1	1.5	1.6	1.2
VS (% of TS)	86.3	67.2	49.8	63.9	47.9
Total N (g/kg)	2.7	1.3	0.6	0.5	0.3
Organic N (g/kg)	2.2	0.8	0.2	0.4	0.2
NH_4^+-N (g/kg)	0.5	0.4	0.4	0.2	0.1
Total C (g/kg)	36.3	8.1	5.0	5.1	3.4
C/N ratio	13.3	6.3	8.5	9.4	11.0
P (g/kg)	0.37	0.20	0.15	0.74	0.39
K (g/kg)	1.37	1.25	1.19	1.14	0.96
Mg (g/kg)	0.24	0.19	0.08	0.06	0.09
Ca (g/kg)	1.77	1.23	0.06	0.07	0.03
Na (g/kg)	0.52	0.51	2.09	1.27	1.70
S (g/kg)	0.24	0.08	0.05	0.06	0.04

F Populärvetenskaplig sammanfattning

Odlad fisk utgör den snabbast växande källan till animalisk föda i världen idag, och kommer att fortsatt vara en viktig källa till föda för världens växande befolkning under kommande år. Samtidigt har mängden vildfångad fisk stagnerat sedan 90-talet, något som innebär ett problem inte bara i ljuset av fler munnar att mätta utan även för fiskodlingsindustrin. Eftersom vildfångad fisk är en viktig ingrediens i fiskfoder riskerar en ökad produktion av odlad fisk att sätta ytterligare press på redan hårt utnyttjade fiskbestånd.

För att råda bot på detta och säkerställa tillgängligheten av odlad fisk även i framtiden, pågår idag en jakt efter hållbart producerade alternativa proteinkällor för fiskfoder. Mikrobiellt protein, ibland kallat SCP (för Single Cell Protein), som består av mikroorganismer såsom bakterier och jäst, skulle kunna utgöra en sådan alternativ proteinkälla. För att producera SCP krävs dock ständig tillgång till energirikt odlingsmedium. Exempel på sådana odlingsmedier är naturgas och biprodukter från raffinering av socker, melass. Tidigare försök att odla mikrober som proteinfoder har dock inte visat sig vara långsiktigt ekonomiskt hållbara.

Målet för denna uppsats var att undersöka om biogassubstrat, bestående av hushållsavfall och gödsel, skulle kunna utgöra ett lämpligt odlingsmedium för framställning av foderjäst. Biogassubstrat är ett billigt medium som finns i rikliga mängder överallt där människor och jordbruk finns, och skulle kunna lämpa sig mycket väl för produktion av mikrobiellt protein.

Då man framställer biogas rötas restprodukter av olika slag i en biogasreaktor, där en rik flora av mikroorganismer bryter ned materialet och producerar metan. Metanen kan sedan lagras och användas för att driva fordon eller framställa energi. Resterna från processen kan spridas på åkrar som s.k. biogödsel eller rötrest.

Genom att kombinera jästodling och biogasframställning skulle man kunna få ut både foderjäst, biogas och biogödsel. Då biogasprocessen aldrig når fullt utnyttjande av materialet, dvs. organiskt nedbrytbart material finns kvar i rötresten, finns utrymme för ytterligare nedbrytning av materialet.

I denna uppsats undersöks hur jästodling och biogasproduktion skulle kunna samexistera. Jäst odlades på biogassubstrat, och efter att den resulterande jästmassan skilts från mediet producerades biogas från återstoden. Genom att mäta hur mycket gas som producerades kunde inverkan av jästodling på biogasproduktionen mätas. Ett flertal jäststammar undersöktes, och de som växte bäst på mediet odlades i ett större försök. Resultaten från detta försök visade att både utbyte av jäst samt produktionen av biogas på återstoden var starkt beroende av vilken jäststam som användes.

En jäststam visade sig mycket kapabel att bryta ned biogassubstratet, och

producerade nästan 15 g torr jästmassa per liter substrat. En annan jäststam producerade endast 7 g per liter, men visade sig ha en positiv inverkan på biogasproduktionen.

Sammanfattningsvis kan man säga att jästodling och biogasproduktion skulle kunna vara mycket väl lämpade för att samexistera, men att valet av stam är väldigt viktigt för att uppnå god produktion och lönsamhet i processen. Produktion av foderjäst på biogassubstrat skulle kunna utgöra en del av lösningen på framtidens proteinbehov inom fiskodlingsindustrin, och därmed även för framtidens livsmedelssäkerhet.