Water Practice & Technology Vol 15 No 2
doi: 10.2166/wpt.2020.036

# Acid whey treatment and conversion to single cell protein via aerobic yeast activated sludge

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## Abstract

In this study, a synthetic acid whey was made to mimic acid whey produced during cheese manufacture. A mixed yeast culture, primarily *Vanrija albida*, was used to degrade the wastewater and produce a single cell protein (SCP). The system was operated in batch mode at high and low loading rates. The COD-use efficiency of the biomass was 93 and 85% at the high and low loading rates, respectively. The pH was maintained at 3.5 to prevent bacterial contamination of the system. High loading and high oxygen transfer efficiency indicate that a full-scale yeast system would probably offer significant cost savings over both aerobic and anaerobic bacterial systems. The biomass produced has the potential to be a valuable commodity. The biomass amino acid profile was good with respect to the FAO protein nutrition guidelines for various farmed livestock species.

**Key words:** acid whey, single cell protein, *Vanrija Albida*, wastewater, yeast, yeast activated sludge

## HIGHLIGHTS

- Novel method of degrading acid whey wastewater.
- Described method will allow a significant reduction in the size of reactors needed to degrade this high strength industrial wastewater.
- Biomass produced by the method has excellent characteristics and should be suitable for use as livestock feed supplement.
- This research identified a specific species of yeast that was particularly suited to degradation of this material but has received little attention in past research.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| ATC | Automatic Temperature Control |
| COD | Chemical Oxygen Demand |
| DNA | Deoxyribonucleic Aid |
| DO | Dissolved Oxygen |
| F/M | Food/Microorganism Ratio |
| FAO | Food and Agricultural Organization (United Nations) |
| GRAS | Generally Regarded As Safe |
| MLSS | Mixed Liquor Suspended Solids |
| PID | Proportional Integral Differential |
| RPM | Revolutions per Minute |
| SCP | Single Cell Protein |

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SMP Soluble Microbial Products
TKN Total Kjeldahl Nitrogen
TSS Total Suspended Solids
USDA United States Department of Agriculture
YNB Yeast Nitrogen Base

## INTRODUCTION

The manufacture of dairy industry products such as yogurt and cheese yields liquid waste known as whey. There are two types: sweet whey and acid whey, whose typical physical and biochemical properties, as reported in the literature, are given in Table 1.

**Table 1** | Typical whey parameters given in the literature – e.g., Lievore *et al.* (2015), Pescuma *et al.* (2015), Moeini *et al.* (2004), Yadav *et al.* (2014)

| Parameter | Sweet whey | Acid whey |
|---|---|---|
| COD (mg/l) | 80,000 | 60,000 |
| BOD (mg/l) | 60,000 | 40,000 |
| Lactose (%) | 6 | 4 |
| Protein (%) | 1 | 1 |
| Lactic acid (%) | Trace | 0.8 |
| pH | 6.5 | <5 |
| Salts (mg/l) | 1,000 | 1,000 |

This paper focuses on the treatment of acid whey, which is problematic due to its very high COD compared to most food-processing wastewaters. Treatment of whey can be accomplished by:

- Aerobic treatment
- Anaerobic treatment
- Land application
- Microbial fermentation
- Reuse/mixing with other food products
- Single cell protein production

Aerobic wastewater treatment processes are uncommon because of their very large physical size (primarily tankage) and high energy demand relative to other processes. When used, aerobic treatment is generally added to polish effluent following other processes – for example, controlled feed to municipal waste treatment digesters, and anaerobic treatment (Chatzipaschali & Stamatis 2012; Prazeres *et al.* 2016).

Direct (untreated) land application is problematic due to the high organic load, which can cause changes in soil structure and degrade soil quality (Prazeres *et al.* 2016). Anaerobic treatment has the benefit of producing biogas, which has monetary value; the payback period after installation is typically between 5 and 16 years (Chatzipaschali & Stamatis 2012).

Single cell protein (SCP) production using whey as substrate has been accomplished using yeast. The most common genus used is *Kluyveromyces* (Ghaly *et al.* 2005; Yadav *et al.* 2014; Pescuma *et al.* 2015). Its advantage is that it already has a generally regarded as safe (GRAS) rating from the United States Department of Agriculture (USDA) and the product can be used as an animal feed supplement. A disadvantage is that it sometimes ferments lactose instead of turning it into biomass, which is undesirable in SCP production.

A variety of other microorganisms can also convert whey to useful byproducts, including: ethanol, butanol, hydrogen, lipids, polyhydroxyalkanoates, lactic acid, lactobionic acid, exopolysaccharides,

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galactooligosaccharides, ribonucleotides, ergosterol, propionic acid, and vitamin $B_{12}$ (Nemeth & Kaleta 2015; Pescuma *et al.* 2015).

The most common methods of whey wastewater treatment and disposal are anaerobic digestion and land application. The anaerobic digestion sludge does not degrade the soil but, as for most land application processes, disposal is linked to agricultural timing and regulatory regimes – for example, nutrient loading limits, and the timing of planting and harvesting.

SCP production is potentially interesting because it is relatively simple and the biomass yield is usable as animal feed or to produce yeast extract. Converting the whey to SCP also reduces transport, handling, and disposal costs, as well as creating potential revenue streams in the process residuals. Additional advantages are that yeasts thrive in the acidic conditions created as whey degrades and can tolerate the very high volumetric and F/M loading, as well as rapid changes in osmotic pressure. As a result, yeast treatment systems are:

- Significantly smaller than alternative processing (treatment) systems, thereby reducing capital costs, and
- Easy to operate.

In this study a mixed yeast culture was introduced and acclimated to degrade a simulated acid whey wastewater in a bench-scale reactor. The dominant species was *Vanrija albida*, which yielded more than 99% of the DNA in the sample analyzed. Conditions in the reactor were manipulated to suit, and thus select, species that can metabolize lactose aerobically (no fermentation) with limited, if any, micronutrient addition and without vitamin addition. Reactor operating conditions were varied to test high- and low- loading scenarios. The resultant biomass amino acid profile is compared to FAO guidelines for various livestock feed requirements, and operating parameters, oxygen uptake curves, nutrient requirements, cell yield, and effluent quality are evaluated.

## MATERIALS AND METHODS

### Acid whey formula

The acid whey mixture was designed to mimic the profile presented in Table 1. The feedstock was composed of 40 grams of lactose, 10 of whey protein blend, and nutrient made up to 1 liter with distilled water. Two dosing schemes were used to create 'high' and 'low' loading conditions. In high load condition, reactor mixed liquor was discharged until 200 ml of mixed liquor per liter of reactor volume remained. 800 ml of acid whey per liter of reactor volume was added per batch. In the low loading condition, reactor mixed liquor was discharged until 500 ml of mixed liquor per liter of reactor volume remained. 500 ml of acid whey per liter of reactor volume was added. A diagram of the waste and fill scheme is shown in Figure 1. The whey protein blend used was Optimum Nutrition 100% Gold Standard Whey (chocolate). Reactor loading conditions are presented in Table 2.

### Nutrient formula

The nutrient formula was developed over the course of the research. The initial version came from Frigon & Liu (2016), the final formula is provided in Table 3.

### Culture formation/enrichment

Initially, lactose-consuming, non-fermenting yeasts were investigated. *Fellomyces penicillatus* was chosen as the best potential candidate (Kurtzman *et al.* 2011), after evaluation, although other choices were/are possible.

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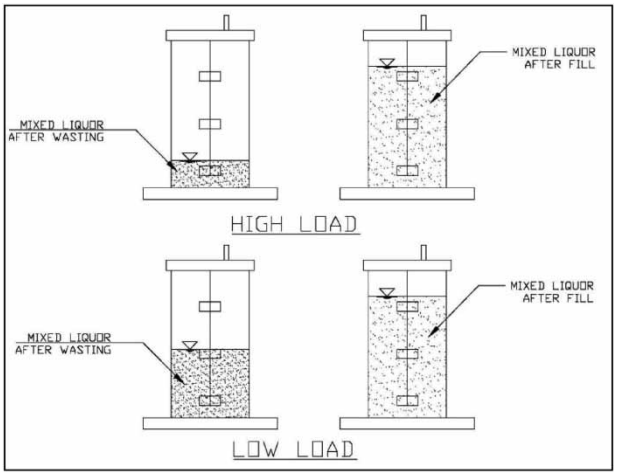


**Figure 1** | Schematic showing waste and fill method for high and low loading conditions.

**Table 2** | Reactor high- and low- loading conditions for treating acid whey

| Parameter | High-load | Low-load |
|---|---|---|
| COD (mg/l) | 65,000 | 32,000 |
| Lactose (%) | 3.2 | 2 |
| Protein (%) | 0.8 | 0.5 |
| pH | 3.5 | 3.5 |

**Table 3** | Final nutrient formula for treatment of acid whey with yeast biomass

| Chemical | Element | Nutrient formula (g/l) | Chemical dose (mg/l) | Element dose (mg/l) |
|---|---|---|---|---|
| $KH_2PO_4$ | P | 47 | 2,820 | 643 |
| $MgSO_4$ | Mg | 47 | 2,802 | 276 |
| $ZnSO_4$ | Zn | 0.05 | 3.00 | 0.68 |
| $CaCl_2$ | Ca | 0.10 | 6.00 | 2.17 |
| $MnCl_2$ | Mn | 0.10 | 6.00 | 2.62 |
| $FeCl_2$ | Fe | 0.10 | 6.00 | 2.06 |
| $(NH_4)6Mo7O24$ | Mo | 0.02 | 1.20 | 0.69 |
| $CuSO_4$ | Cu | 0.02 | 1.20 | 0.48 |
| $CoCl_2$ | Co | 0.02 | 1.20 | 0.54 |

A pellet of freeze-dried yeast cells (ATCC# 32128, Manassas, VA, USA) was grown in a fermenter (Major Science, FS-02 series, Saratoga, CA, USA) using the standard yeast nitrogen base (YNB) with glucose as a carbon source (Kurtzman *et al.* 2011). Once the culture was enriched, a mixed culture of freeze-dried yeast developed during previous high strength wastewater research (Frigon & Liu 2016) was added to enhance the ability of the biomass to consume wastewater constituents and enhance contaminant removal (Moeini *et al.* 2004).

The biomass was acclimated from YNB/glucose to the nutrient/acid whey formula over a week. The qualitative oxygen uptake curves revealed that the biomass was slightly inhibited and analysis showed that all phosphorus in the system had been used, the phosphorus concentration used was doubled. Two weeks after the reactor was started, yeast extract was removed from the nutrient

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formula to check its effect on reactor performance. Adaptation to the change took about 2 days but the biomass remained viable. After observing very high nitrogen effluent concentrations, nitrogen was removed from the nutrient formula (except the very small amount associated with the molybdenum micronutrient).

Once reactor operation was stable with the mixed culture and modified nutrient formula – after about two weeks – the system was considered mature and testing began. Stable operation was identified by consistent effluent COD levels, moderate amounts of residual phosphorus and nitrogen, no apparent inhibition in oxygen uptake, and stable biomass composition under microscopic examination.

The reactor was first operated at the high loading rate and tests were performed for that condition. After those tests were complete, the reactor was operated at the low loading rate condition for two weeks, until effluent conditions and reactor operation were stable. The low loading tests were then conducted.

### Fermenter operation

The fermenter was operated in batch mode. The 3-liter reaction vessel was operated at 2 liters for all tests and reaction progress was tracked using the mixing speed. Vessel mixing speed is directly related to oxygen uptake rate because higher mixing speeds increase the oxygen transfer rate into the bulk liquid. During high-load tests, the reactor was fed every 36 hours, during low-load tests, every 20 hours.

The fermenter controlled DO, mixer RPM, pH, and temperature, and recorded them once per minute. Table 4 shows the fermenter set points. The fermenter's pH PID control kept the pH within $+/-0.1$ of the set point. Air was fed into the fermenter at a constant rate of 4 L/min, the flow being controlled by a rotameter. The oxygen transfer rate was regulated by a mechanical mixer with automated speed control and DO was near constant. Minimum mixing speed was set at 250 rpm but, when the DO concentration moved outside the set points, the mixing speed was automatically increased/decreased as needed to meet oxygen demand.

**Table 4** | Reactor operating conditions

| Parameter | Value | Variability |
|---|---|---|
| pH | 3.5 | +/− 0.1 |
| DO (%) | 25 | +/− 5 |
| DO (mg/L) | 2.1 | +/− 0.4 |
| Air flow (L/m) | 4 | +/− 0.0 |
| Air flow (L/L/m) | 2 | +/− 0.0 |
| Temperature (°C) | 25 | +/− 0.1 |

### Oxygen uptake curves

Oxygen uptake curves are generally developed using a respirometer or similar instrument to quantify the amount of oxygen consumed by the biomass. This could not be done with the fermenter used in this study. The fermenter could vary the mixing speed to meet a DO set point. Recording changes in mixing speed enables a qualitative measure of oxygen uptake rate to be obtained.

### COD, phosphorus, TKN, and total nitrogen tests

COD, phosphorus, TKN, and total nitrogen tests were all performed using a DRB200 reactor and DR3900 spectrophotometer (Hach, Loveland, CO, USA). All tests were performed in duplicate,

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and effluent samples were centrifuged at 4,000 rpm for 10 minutes and passed through a 0.45 micron filter prior to analysis.

### TSS tests

TSS tests were performed using Standard Method 2540 D (APHA 2012).

### Specific gravity tests

Specific gravity was tested using an automatic temperature control (ATC) salinity refractometer (ADE Advanced Optics BCBI9177).

### Microscopic examination

Microscopic examination was performed with a standard light microscope.

### Amino acid and DNA analysis

Amino acid and DNA analyses were performed on the biomass. 50 ml aliquots were taken at the end of the reaction time, washed three times with distilled water, and sent to MtoZ Biolabs (Boston, MA, USA) for analyses.

## RESULTS AND DISCUSSION

### Specific gravity

The specific gravity of the filter liquid in the bioreactor at the beginning of the high-load batch reaction was 1.037, somewhat higher than sea water (1.023); by the end, it was 1.008. The equivalent values for the low-load condition were 1.020 and, again, 1.008 – see Figure 2.

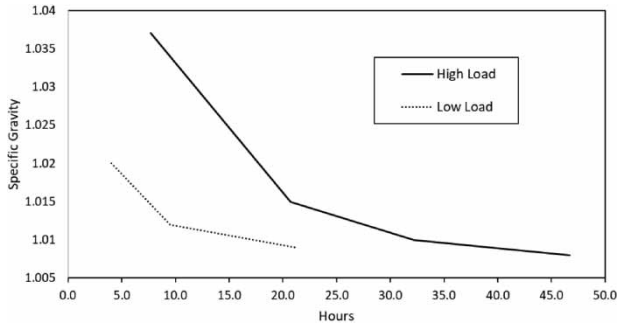


**Figure 2** | Reactor effluent specific gravity vs reaction time. (*Note*: specific gravity at time = 0 was not determined).

### COD loading

COD loading for the reactor was high both volumetrically and by mass. Table 5 presents the loading conditions during this work, as well as typical aerobic and anaerobic reactor loadings. Volumetric loading rates were calculated on the basis of 36-hour reaction time for the high-load condition and 20-hour reaction time for low-load, the times being derived from the oxygen uptake curves.

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**Table 5** | Reactor loading conditions in this study, and typical loading values for aerobic and anaerobic reactors

| Reactor | Volumetric loading (g-COD/L/day) | Mass loading (g-COD/g-biosolids) |
|---|---|---|
| Acid whey high-load | 43.4 | 19.5 |
| Acid whey low-load | 39.0 | 7.8 |
| Typical aerobic | 0.9 | 0.4 |
| Typical anaerobic | 12.0 | 0.5 |

## COD removal, yield, and nutrient uptake

COD removal in the high- and low- load reactors was 97 and 85% respectively, with corresponding final effluent COD concentrations of 4,835 and 4,720 mg/L.

Figure 3 shows changes in COD, nutrient, and MLSS concentrations vs. time, and their high- and low- load influent and effluent concentrations are given in Table 6.

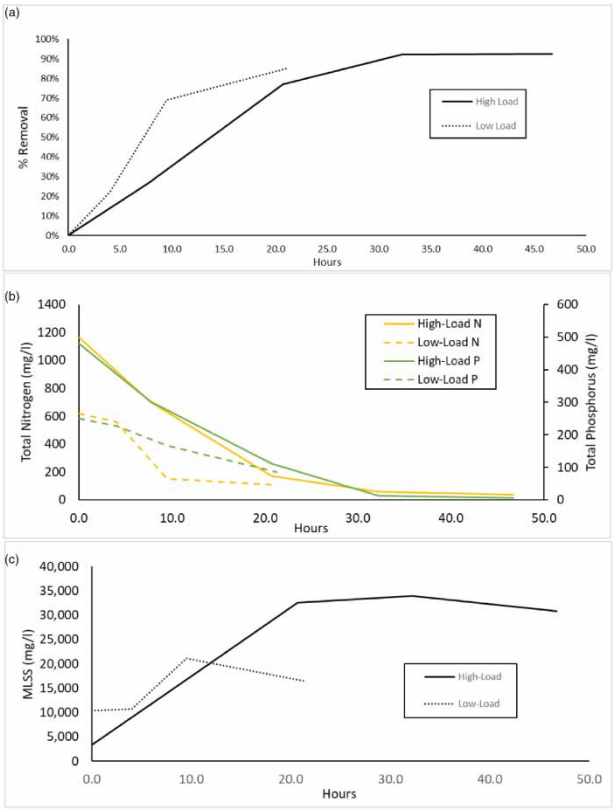


**Figure 3** | (a) COD removal vs. time for high- and low- load conditions. (b) Nitrogen and phosphorus uptake for high- and low-load conditions. (c) Change in biomass concentration in high- and low- load conditions vs. time.

**Table 6** | Summary high- and low- load reactor influent and effluent

| Reactor | Influent COD (mg/l) | Effluent COD (mg/l) | COD reduction (%) | Influent N (mg/l) | Effluent N (mg/l) | Influent P (mg/l) | Effluent P (mg/l) | Yield (g-TSS/g-COD removed) |
|---|---|---|---|---|---|---|---|---|
| High Load Reactor | 65,050 | 4,834.5 | 93% | 1,165 | 39.75 | 480 | 6.6 | 0.51 |
| Low Load Reactor | 32,362.5 | 4,720 | 85% | 617.5 | 107.6 | 249 | 86.1 | 0.39 |

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### Amino acid profile

The biomass amino acid profile was evaluated under both high- and low-load conditions. Table 7 presents the amino acid profiles for both high- and low-load conditions, as well as the ideal profiles for feed for various types of livestock. The livestock-related profiles are based on the lysine requirements according to FAO guidance (lysine requirement = 100) (Miller 2003).

**Table 7** | Amino acid profiles of biomass from high- and low- load reactors, as well as for ideal feed profiles for several animals, based on lysine content

| Amino acid | High loading profile | Low loading profile | Pig ideal profile | Chick ideal profile | Salmonid ideal profile | Cat ideal profile |
|---|---|---|---|---|---|---|
| Asparagine | 67 | 124 | | | | |
| Threonine | 77 | 83 | 64 | 67 | 42 | 90 |
| Serine | 70 | 41 | | | | |
| Glutamic acid | 99 | 110 | | | | |
| Proline | 73 | 71 | | 44 | | |
| Glycine | 81 | 76 | | 65 | | |
| Alanine | 172 | 111 | | | | |
| Valine | 77 | 76 | 74 | 77 | 46 | 80 |
| Methionine + Cysteine | 80 | 56 | 51 | 72 | 50 | 90 |
| Isoleucine | 70 | 67 | 57 | 67 | 42 | 65 |
| Leucine | 145 | 127 | 114 | 109 | 75 | 150 |
| Tyrosine + Phenylalanine | 138 | 128 | 17 | 105 | 110 | 130 |
| Lysine | 100 | 100 | 100 | 100 | 100 | 100 |
| Histidine | 38 | 33 | 36 | 32 | 33 | 35 |
| Arginine | 101 | 80 | 40 | 105 | 88 | 150 |
| Tryptophan | [a] | [a] | 17 | 16 | 13 | 15 |

*Note*[a]: Tryptophan was not determined.

### DNA analysis and biomass composition

The system's operation enabled very selective growth of particular yeast species, which is important if the species is cultivated for resale/reuse. In this case, it was possible to maintain a consistent culture for a sustained period under varying loading and operating conditions. Table 8 shows the proportional breakdown of the nine most abundant species determined by the DNA analysis.

## DISCUSSION

The reactor was operated under two loading conditions to test changes in reaction time, SCP yield, and final effluent quality.

High specific gravity results were returned during the early part of batch operation. High specific gravity correlates to high osmotic pressure in cells in the water. High osmotic pressure is important for two reasons:

1. The osmotic pressure in the reactor changes significantly over the reaction period. This is due to dissolved solids removal as the yeast consumes whey, lactose and other solutes. Even moderate salt concentration/osmotic pressure changes over short periods (rapid) have been shown to inhibit bacterial systems (Kargi & Dincer 2000).

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**Table 8** | Nine most abundant yeast species in the biomass, determined by DNA analysis of the sludge

| Species | Percentage |
|---|---|
| *Vanrija albida* | 99.07% |
| *Solicoccozyma aeria* | 0.15% |
| *Mortierella alpina* | 0.12% |
| *Plectosphaerella cucumerina* | 0.06% |
| *Neocosmospora rubicola* | 0.05% |
| *Alternaria alternata* | 0.04% |
| *Thanatephorus cucumeris* | 0.02% |
| *Fusarium oxysporum* | 0.02% |
| *Apiotrichum veenhuisii* | 0.02% |

2. High osmotic pressure favors yeast cell growth kinetics. Dan *et al.* (2003) showed that when salt concentrations exceeded 25 g/l (specific gravity of ~1.020), yeasts grew faster than bacteria.

Reactor loading conditions are important because they affect the reactor size. High-load reactors are smaller than low-load reactors, which reduces their capital and operating costs, and space requirements. As the yeast can withstand high loading, full-scale reactors can be significantly smaller than other biological treatment systems.

Run times exceeding the time taken to consume the primary substrate, as determined by mixing speed (aeration rate), did not lead to significant additional COD removal. The lower removal efficiency of the low-load reactor may be deceptive as the residual COD in both reactors probably comprised soluble microbial products (SMP) produced by the biomass (enzymes, etc) while consuming the acid whey. As such, the COD residual reflects biomass activity rather than remaining unconsumed substrate. After yeast separation from the mixed liquor for reuse, the reactor effluent is likely to require polishing, perhaps in a bacterial system, to remove further COD prior to discharge.

Nitrogen in the system came entirely from the acid whey. The high-load condition removed nitrogen somewhat better than the low-load condition, which reflects the greater biomass production. The high nitrogen content means that no additional nitrogen needs to be added for effective treatment, although some secondary treatment may be needed to remove nitrogen remaining in the effluent.

In early tests, observation of the mixing speed curves indicated inhibition due to inadequate phosphorus content and its concentration was doubled later in the testing. This indicates a need for phosphorus addition to achieve optimal treatment of acid whey when using yeast.

SCP yields for the high- and low- load conditions were 0.51 and 0.39 g-biomass/g-COD-consumed, respectively. The high-load condition is thus superior for SCP production. Higher yield under high-load conditions was also observed by Ghaly *et al.* (2005). The MLSS concentration fell slightly in both reactors over time, when the consumable substrate was used. This type of mass loss has been observed previously in yeast-related studies (Frigon & Liu 2016) and might have been caused by consumption of stored reserves and/or some degree of apoptosis.

The reactor was operated at low pH to minimize bacterial growth. Initially, reactor pH was 4.5, under which condition the yeast grew well but bacterial growth was also significant. This was apparent from both the activity in the reactor, as observed by mixing speed, and from microscopic inspection of the biomass. When reactor pH was maintained at about 3.5, there was no noticeable bacterial activity. No attempt was made to produce a sterile culture other than operational changes that favor yeast growth:

- low pH
- high initial volumetric and mass loading
- batch treatment

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Yeast cells are large, so the health of the biomass and bacterial infection can be determined readily by microscopic inspection. Figure 4(a) shows yeast biomass with considerable bacterial contamination (during early reactor operation). Figure 4(b) shows healthy yeast biomass with little bacterial contamination.

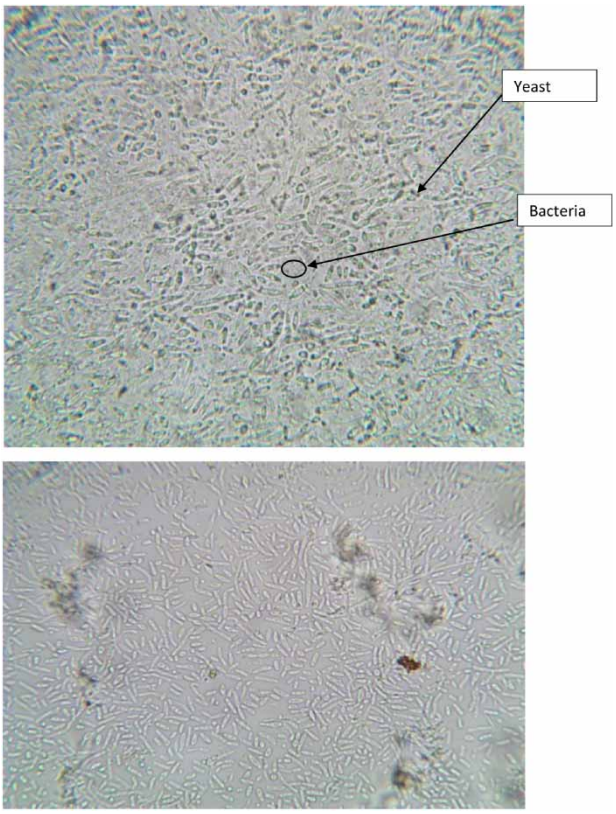


**Figure 4** | (a) Early acid whey reactor biomass with significant bacterial contamination. (b) Later acid whey reactor with no apparent bacterial contamination. (Both images – 400× magnification, 10× dilution).

Reactor mixing speed (rpm) was an effective measure of biomass activity. Reactor mixing speed dropped as oxygen use declined. Once mixing speed declined to the lowest set point, the reaction was essentially finished. In these tests, mixing speed was used but at full-scale the blower capacity needed to maintain some DO could be used in a similar manner to determine when the batch reaction was finished. Yeast cells prefer high loading and die/lyse when starved, so it is important to monitor the reaction as part of the operation and stop the batch once there is no more reaction. Cell biomass lysis results in loss of valuable biomass and increases the effluent's COD.

Figure 5 shows the mixing speed plot from a 'high-load' batch. It is similar to a respirometry curve. Although it is not as precise, it provided qualitative data about how the reactor was operating. It was easy to tell, for instance, when bacterial contamination was present, because bacterial oxygen uptake is much higher than for the yeast biomass. The maximum mixing speed for the yeast biomass was approximately 700 rpm but, when there was bacterial contamination, the maximum rose to approximately 1,100. Dan (2001) noted that air diffuses more rapidly into yeast biomass and suggested that that this might be due to yeast floc properties, although he did not identify any specific mechanism or reason for this. The reduction in energy used for yeast system aeration is likely to reduce operating costs compared to aerobic bacterial systems.

High loadings, both volumetric- and mass- based, are needed for yeast to remain dominant in biological reactors. This type of loading scheme generally can only be accomplished by batch reactor. Some researchers have tried to operate continuously fed reactors, but COD removal efficiency and

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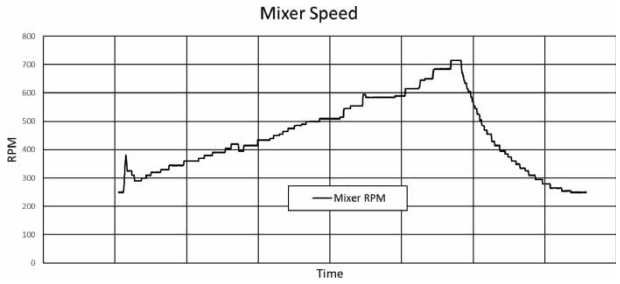


**Figure 5** | Mixer speed vs. time with increasing oxygen demand in the reactor.

yield were poor (Yadav *et al.* 2014). In this study, high- and low- load batch schemes offered high COD removal and similar effluent quality. The advantage of the low-load scheme was a reaction time of 20 hours compared to 36, but the biomass yield and amino acid profile were better under the high-load scheme.

The biomass produced during whey degradation is potentially valuable as an animal fodder supplement. The high- and low- load condition amino acid profiles are close to ideal for both pigs and chickens, and look promising for various fish species (only salmonid-related data are presented here). The profiles indicate that the yeast cell proteins are well suited to meeting the nutritional needs of many animals. For the yeast used in this research to be used as fodder, it would need certification as GRAS. A number of yeast species can degrade lactose, however, and might be viable alternative candidates for acid whey degradation. Another potential market for the biomass is yeast extract production. Yeast extract has high value at either lab or industrial quality and the volume that could be produced is quite high due to the strength of the wastewater.

The DNA results show that *Vanrija albida* was the dominant yeast in the culture, comprising over 99%. The species has not been investigated in the literature except as a passing note as being found in soil (Buzzini *et al.* 2017). The Mycobank database identifies *Sporobolomyces albidus* as a synonym, further tracking of which in Kurtzman *et al.* (2011) leads to *Cryptococcus ramirezgomezianus* as another synonym.

## CONCLUSIONS

- Using yeast to degrade acid whey from dairy manufacturing is viable.
- Yeast reactor loading can be much higher than that of bacterial reactors on both volumetric and F/M basis, reducing reactor size significantly.
- Biomass produced by yeast has a good amino acid profile and may be suitable in livestock feed.
- *Vanrija albida* was the dominant species under the reactor conditions in this study and may deserve attention in relation to wastewater treatment.

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