Chapter 15

Visual Bart Reader VBR I and II systems

15.1 Introduction

In this "modern era" when virtually all devices have to include time saving techniques that reduce monitoring costs and improve precision and reliability. In DBI these concerns are taken very seriously and this chapter addresses the various systems and software that have been developed and is available from DBI.

There are two systems that can be used for the time lapse photography of the Bart testers when they are being incubated. These are known as the visual Bart reader (VBR) systems. Both VBR systems employ two rows of nine testers which are illuminated from below. There are therefore a total of eighteen testers within a VBR system. These are monitored using a time lapse camera that records an .jpg image every fifteen minutes. There are two versions. VBR I is the basic set up that would normally operate at room temperature (generally recognized to be 22±2^{o}C). VBR I can be placed in a temperature controlled room but the upper temperature limit is 45^{o}C. VBR II has the advantage of the rack of illuminate Bart testers being placed with a temperature controlled incubator that will function at room temperatures up to 62^{o}C. If the VBR II is placed in a cold room then it is possible to get down to 4^{o}C. VBR II set is shown in Plate 15.1 and 15.2. %CBR software interpretation is shown in Plate 15.3.

Software exists for the Bart testing in two distinct bundles. The first software described below is the percentage confirmatory bacterial reduction (%CBR) and is primarily linked to the development of a new approach to the more rapid testing for biochemical oxygen demand in treated effluents to be released into the environment. Here the %CBR software can only be used with the HAB- or BOD- testers. The other software package is called the VBR software and is for the interpretation the reactions and activities from the SRB-, IRB-, SLYM-, HAB-, DN-, and the FLOR- testers. Information on the VBR software is available at no charge by contacting sales@dbi.ca. %CBR software can also be obtained in this manner but instructions on its use is given below as sections 5.2 to 5.9.

15.2 Preparation of the HAB- or BOD- tester for %CBR testing

It is recommended that latex gloves be worn during the setting up of the laboratory version of the HAB- or BOD- Bart tester to reduce the risk of contamination. Follow the sequence of activities as described below.

(i) Write the sample information with a fine tipped permanent black marker above the fill line so that it can be easily viewed by the operator and/or the VBR I system. Note that the VBR I system is required to incubate the temperature to 22±1^{o}C as well as the time lapse camera and software. Note 22±1^{o}C is the recommended temperature for the confirmatory bacterial reductions test. If the rom for the testing cannot be maintained at 22±1^{o}C then the VBR II system can be employed to ensure that those temperatures are maintained. In the event that the room for the testing is commonly above that recommended temperature then the VBR II unit can be used installed within a larger refrigerator or cooled room set to no higher than 15^{o}C. The incubator will now maintain the desired temperature of 22±1^{o}C.

(ii) Unscrew the inner (dark or light blue) cap from the HAB- or BOD- tester (respectively) and place the cap down on a clean surface without turning it over. Note also that the contents of the tester are now exposed to possible contamination from the outside environment and so the next steps should be done quickly. Unscrew the cap from the sample container and slowly pour sufficient sample (to be tested) into the inner tester vial to bring the water level up to the fill line indicating 15±0.25mL of sample has been added. The ball will float up on the rising sample water column extending up to the fill line. When pouring or pippetting samples into the uncapped tester vial, every effort should be made to direct a stream of the sample water over the center of the ball rather than allowing it to trickle down the side of the tester. Maximum tolerance for error when filling the tester is 5% so that the amount of water sample being tested falls within the range of 14.75 and 15.25mL. It should be noted that the sample container retains a headspace of air over the sample so that some oxygen will diffuse down into the liquid sample during testing.

(iii) Once the tester has been charged to the fill line then immediately screw the cap down on to the tester. Invert the freshly charged vial for 30seconds to allow the methylene blue

indicator (dried in the cap) to begin the diffuse into the sample and turn it blue (oxidative). To ensure that the blue indicator is evenly mixed into the sample and now rotate the tester three times using a gentle wrist action.

(iv) The charged (blue) tester can now be placed in the appropriate slot on the VBR I rack. Here for wastewater testing it is suggested that triplicate testing be performed. In this case PE would be placed in A1, 2, and 3 slots (upper left) and FE in the B1, 2, and 3 slots (upper central three slots). Other triplicated samples may be placed in triplicate in slots C, D, E or F. Make sure that the sample label always faces towards the camera and so it will be readily recorded on every .jpg image.

(v) It cannot be overemphasised that preparing the testers should be done quickly with precision and immediate loading into the VBR I as quickly as possible. Inadvertent delays in starting can seriously impair accuracy. Once the VBR door is closed with the testers in place then the camera should be started by moving the rotary mode switch from "SET UP" to "AUTO" (see section 15.5). Remember to move quickly out of the field of view to ensure that the first image is clean and does not include the odd arm, hand or eye! Remember to make sure that the memory card inserted into the camera has been wiped clean of all data and certainly contains no jpeg images (.i.e. is empty). The time lapse camera will automatically take the first image on the memory card to be the first image and all subsequent images will be set at 15 minute intervals.

Positive reactions commonly occur in wastewater using the HAB- or BOD- testers will go positive in less than 30 hours with UP reactions in less than thirty hours at $28\pm1^{o}C$. As the testers take longer to go positive then the active populations detected becomes smaller. Time lapse is therefore inversely related to population and so a sample taking 30hours to go positive is a very small population.

15.3 Comparisons with the standard BOD5 protocol

In 2002 a draft document was prepared for the Canadian Environmental Technology Verification but was not submitted due to incompleteness of the trials to define precision. In August 2006 and American ETV document was prepared in parallel with the completion of those trials. In summary this document included the following findings that are still relevant in 2013:

- Seeding of bacteria for the calibration and confirmation of precision was developed although secondary testing (2010 – 12) has revealed that the primary influent (PE) from a sanitary municipal WWTP through dilution can be successfully used for this purpose.
- Correlations between BOD5 and the predicted bacteriological populations achieved using linear regression analysis where R^2 correlations ranged between 0.941 and 0.987. However this varied with each WWTP and led to the conclusion (2012) that comparison should be made between the Primary influent and the sample (e.g. final effluent) being tested at the same time to determine the percentage change (commonly reduction as %CBR) in the bacterial populations from the PI to the sample under test. This was not considered in the previous studies (1996 to 2005).
- Incubation temperatures for convenience has been set at room temperature (22 ± 2^oC) and it was found by thermal gradient analysis of time lapses from ranges as low as 4^oC to as high as 45^oC that there was less variation occurring at 22 ± 1^oC. This temperature generates slower growth than can be achieved at 28 ± 1^oC but initial findings indicate that there is better precision at the lower temperature but with the disadvantage of longer incubation times.
- Sample storage conditions were found to be critical and the recommended protocol was to have the samples come up (or down) to room temperature (22 ± 2^oC) before beginning the test with the storage time should not normally exceed four hours before the start of testing.
- Potential of toxic agents in the samples. It has been found that the PI for the two WWTP studied have populations that range from 4 to 78billion pac/mL. If the PI is found to have a population of less than one billionpac/mL this could be taken as the threshold “marker of concern” since there would be likely to be toxicity affecting the bacterial population. This threshold would be lower than that that which could occur through dilution due to

excessive precipitation or with snow melt run off. This check would be specific for each WWTP and would be affected by environmental; conditions.

- Nitrification inhibition was found to cause a 3% decline in populations in the PI. Since the protocol calls for no more than 30 hours of incubation it is highly probable that the nitrifying bacteria would not yet have become sufficiently active (generally there is three day start-up period needed before this is a significant concern) to generate nitrate which could then interfere with the precision. Due to the short incubation times (<30hours) the inhibitor was considered to be unnecessary CBR test protocol.
- Replicated trials of the BOD5 and the CBR found that there was similar variability found particularly with the percentage variance being: PI 13.7%, PE 9.5%, lagoons ranging from 5.8 down to 2.73% in secondary effluent 2.58 to 3.27% and final effluent had a much better precision averaging at 2.9%.

It is recommend that the VBR I be left permanently in the operable mode creating a minimum power draw of <40watts at 110v AC with a maximum requirement of 940 watts when the incubator heater is on which does not significantly affect the incubation temperature of 22±1^{o}C.

15.4 Operational instructions for the VBR II, time lapse camera and %CBR software

To operate the VBR I then refer to the time lapse camera protocol using the HAB- or BOD- Bart tester and the operation of the standard VBR I supplied software. For the %CBR protocol then the follow the sequence of operations below assuming that the VBR I unit is powered up and operating within the correct temperature range:

1. Obtain wastewater samples for testing and follow the standard methods for testing using HAB- or BOD- testers to generate three (triplicate x 15mL) samples from each sample being tested.
2. Check the time lapse camera is set up correctly for taking time lapse images of the VBR I every fifteen minutes.
3. Confirm that the camera has at least a 4GB memory card in the required slot and that there are no saved images on the card. IT IS ESSENTIAL TO ERASE ALL DATA AND IMAGES FROM PREVIOUS WORK THAT MAY ON THE CARD. Note that these images

can be saved within a designated folder on the computer or on a memory stick before erasing off the memory card.

4. Quickly place all of the charged triplicated HAB- or BOD- testers in the appropriate slots in the VBR I. Remember is important to do this quickly since delays in setting up will compromise precision. It is recommended that PI is placed in slots A1 –A3, and FE be placed in slots B1 – B3. Slide each tester completely into the slot ensuring that the tester has been gently pressed back as far as possible into the slot. Make sure that the camera is set to focus at 13 to 17" to assure images are all in focus. Do not prepare testers using cold samples (at $<19^{o}C$) that have not been acclimated to room temperature since these lower temperatures could significantly affect time lapse data and run the risk of predicting lower populations.
5. Once the testers (minimum six in slots A and B, maximum an additional twelve in slots C, D, E, and F) then power up the camera and the move the switch from "set up" to "auto". The camera will now take the first image (.jpg) at zero time and then takes additional images every fifteen minutes. Note that the camera has been pre-positioned to photograph all of the slots in a precise and standard manner.
6. This testing can continue until all of the HAB- or BOD- testers have reacted (causing an UP or DO reactions where positive detections occur). Once this has happened (commonly within 30 hours) then the camera can be shut down (by moving the switch from "auto" to "set up" and then removing the 4GB memory card from the camera. Note that the VBR I system %CBR software will only interpret images by the interpretation of the data to time lapse (in hours), predicted populations (pac/mL) and thence to the calculation of the confirmatory %CBR reduction as a predicted cell count fraction (as a percentile) in the FE as compared to the PE.
7. Remove all of the testers after the completion of the run for disposal. Take the memory card from the camera and insert it into a suitable slot on the computer into which the %CBR software has been installed and move to interpretation protocol.

15.4.1 Camera Protocol

The camera is precision calibrated for the VBR I and the images do not have any "informational border". Calibration is applied to the setting of the first jpg image at zero time. Specifically the interpretation involves two regions where the pixels are detected that are then used to detect reactions in the HAB – or BOD- tester. These regions are placed 25 and 30mm up from the base of the tester along the vertical mid-point seam. Once the calibration has been adjusted and confirmed then it will remain constant in that particular VBR I system. Additionally calibration is applied to the camera for the following features:

- Focal length set to 13" to 17"
- Time lapse set to every fifteen (15) minutes
- Photo-quality is set to high at MAX 2560 x 1920
- Daily Wakeup and Sleep both set at midnight
- Camera will automatically shut down the LCD display after three minutes to conserve energy
- Time allocated initially is in Eastern Standard Time but that can be adjusted to match the time zone where the VBR I is to be operated
- Recommended time length for camera run (for the %CBR) if not terminated prematurely is 30hours but if the camera is not shut down the images taken after the 120^{th} frame are not used. Where these do occur (in the more effective WWTP) then the populations generated are too low to significantly influence the %CBR being predicted.

Do not use the USB OUT port on the camera since this is not compatible with the %CBR software. The TV OUT port can be used to examine the current picture (.jpg) on the screen. Note that when the camera is in the "AUTO" a status/standby indicator will flash (red) every three seconds to indicate the power is on to the camera and it is working.

The sequence for starting up a VBR I test run is as follows:

1. Insert the memory card into the camera making sure that all data and images have been erased. Failure to do this would mean that the %CBR software would search and find

the earliest loaded image on the memory card and consider that to be that first image for the interpretation and all subsequent images would be fifteen minutes apart.

2. In SET UP mode the camera is in idle with all of the calibrations retained on an internal flash memory. Do not adjust these calibrations unless there is concern that the camera setting may not be correct in which case go the section on confirmation of Camera Settings.
3. When all of the testers have been placed in the VBR I then the testing should be started immediately by moving the lower left dial on the camera from "SET UP" to "AUTO". Make sure that the camera is powered up which causes a red LED light to blink every three seconds. The first picture will be taken in 5 seconds. Close and clip the cover quickly and **move back** so that there is no shadow generated in the jpg image.
4. Camera will go into a passive mode until the next picture is taken. At that due time the camera will "wake up" and after five seconds take the next frame using the camera's calibrated focussing and exposure control to assure even quality for the images.
5. Photographs as .jpg images will be taken every fifteen minutes until either all of the testers are positive (i.e. moved from blue to a clouded clear or pale yellow) in which case the camera can be moved to "SET UP" mode and the memory card removed for interpretation; or the camera could also automatically be shut down (at 30 hours) still retaining the data.
6. Once the operator has moved the camera to "SET UP" mode (either when all tests are positive with the closure operator initiated, or after the 30 hours completion run if some testers are still negative or blue then power down the camera and the memory card can then be withdrawn from the camera slot and moved to the computer.

Camera Settings, it is recommended that the camera be run on the four AA batteries. Manufacturers claim are that these will last for one month before being replaced. Turn the Camera to "SET UP" using the rotary mode switch (lower left) and you can read the battery status. Anything above 70% means there is enough reserve power in the battery for a full run. The camera status would be displayed on the LCD screen (directly below lens). It is possible to check on the settings by going to START UP button and the time lapse comes up for 15 minutes, confirm by the right arrow button and next select PHOTO by pressing the right arrow button.

Next PHOTO Quality is displayed and the correct method uses the 3,264 x 2,448 (MAX) resolution, press right arrow button to confirm. Next the MULTI-SHOTS is presented and that should read "1-SHOT". Press right button to select and then DAILY WAKE and confirm midnight by pressing right arrow. Next come "DAILY SLEEP" and confirm the same time (midnight) by pressing right hand arrow. This is followed by IMPRINT INFO to which the correct response is YES which is then selected by pressing right hand arrow. This is followed by IMPRINT INFO, DATE /TIME and CAMERA NAME. These should remain the same and so continue to press right arrow button until set up complete. Editing should only be done in the event the set-up is no longer appropriate. Refer to the appendixed camera guide for more information. Note the cameras come pre-set for the VBR I and it is not recommended that these be modified.

15.5 Calculation of the %CBR from the project populations.

Measurement of the %CBR therefore involves primarily the factorial comparison of the PI population with the FE population (as predicted active cells per mL generated from the relevant time lapses observed). It should be noted that these reductions can also be calculated for other sample in the treatment stream. Present practices indicate that an effective reduction in the bacteriological activity in the FE to achieve a suitable reduction in biochemical oxygen demands is needed to allow discharge (e.g. <25mg/L or ppm) would involve a minimum factorial reduction of 0.997 (or %CBR of 99.7%). This would use the equation:

$$\%CBR = ((1 - (\text{predicted active population, FE}) / (\text{predicted active population, PI})) \times 100)$$

Note that percentile reductions (%CBR) could be monitored throughout the WWTP process to determine how effectively the different stages in the treatment process are operating. For these percentile reductions for the %CBR then the equation above could be used with the FE would be replaced by the predicted active populations for that stage in the process (e.g. primary effluent, PE; secondary effluent, SE; lagoon one, L1; and clarifier one, C1.).

From the aerated lagoon experience at the aerated municipal sanitary WWTP it has been found that the PE can have %CBR values ranging from +40% down to -20%. Positive %CBR indicates that there has been an increase in the active HAB- or BOD- populations during the primary

treatment occurring from the PI to the PE. In the trials at the aerated WWTP the majority of cases there was reductions in the active populations to give %CBR values of 10% to 99% depending on the stage in the treatment. In a minority of cases there are surges in bacteriological activity which leads the %CBR factorial responses commonly of up to +40%. For the lagoon systems at the trial WWTP there were generally marginal CBR declines in the range 40 to 60% depending upon the lagoon management practices at that time. SE shows a further %CBR decline commonly in the range of -80 to -95% reductions in the active bacterial populations. Clarifiers when applied as a part of the tertiary treatment (e.g. C1) often showed very high %CBR (0.99 to 99.99% but with this range reflecting the tendency for erratic mixing of the treated effluent with other streams. In the FE it has been found with both the aerated and rapid mixed liquor WWTPs that the final %CBR is commonly well below 99.7% indicating that there had been minimally almost a three order of magnitude decline in the populations and the corresponding biochemical oxygen demand is less than 15mg/L.

15.6 Decision Tree

This decision tree is based upon the interpretation of triplicated test data results in the order of the E-tATP determination (see Chapter 14) and then %CBR as the percentile confirmatory bacterial population reductions from the original PI collected at the time of sampling. Testing involves the simultaneous start of the E-tATP determination (minimally of the PI and FE) and %CBR. Respectively, these tests run with intervals of for fifteen minutes between photographs for a maximum of thirty hours. Wastewaters are notorious for generating outlier data since the collected samples are not microbiologically homogenous and often include complex biocolloidal structures which can easily influence results from those particular impacted samples. During the development of researches on the BOD system (over 28,000 sample sets) since 1996 it has been found that on average one in eighteen (5.5%) of the samples generate results that reflect the heterogeneity of those particular outlier samples. The decision tree takes this into account where possible. The decision tree is as follows:

1. Does the %CBR generate a percentile bacteriological reduction value of 99.7% or higher?

   If the answer is YES then this confirms that the sample would be an FE –equivalent and suitable for discharge then go to 5.

   If the answer is NO then go to 2.

2. Has the %CBR given reduction data in the range of 98.0 to 99.6%?

   If YES then the sample should be considered still as a sample incompletely treated (e.g. SE) and then this would require limited further treatment before the FE is to be suitable for discharge. Go back to 1 once the further treatment has been performed and check the %CBR afresh.

   If NO then the sample still has a very significant biochemical oxygen demand loading and should be subjected to further secondary or tertiary treatments as may be considered necessary before going back to 1.

Because of the risk from outliers influencing the outcome of the testing for %CBR it is recommended that triplicated testing be routinely performed to minimise the impact of those outliers and improve precision.

15.7 Statistical Comparison of generated %CBR values and BOD5

In classic determinations of bacterial populations the methodologies have employed some type of agar based technology or rationalised dilution sequence to determine colony presence or activity. The confirmation of BOD reduction by %CBR employs a new concept that involves no dilution, the use of suitable selective culture media, and the determination of the active population through that signal (reaction activity event displayed as an UP or DO reaction) event occurring as the time lapse to that cultural event. The critical signal is the time length (lapse) to the shifting from oxidative to reductive conditions in the HAB- or BOD- tester from the beginning of the test evidenced by the change in the colour in the tester from blue to clear or pale yellow. This time lapse is commonly measured in fractions of an hour with images being generated every fifteen minutes. Time lapse comparisons are made between the PI (as the input primary influent) and the FE (as the final effluents after all treatments have been completed. For the %CBR system the time lapse sets the population size predicted at $22\pm1^{o}C$ using the VBR I tester system coupled to the %CBR software. Reactions are observed using floor lighting employing daylight LED illumination. These reactions are recognised by a shifting of the methylene blue from the oxidised blue form to the clear reduced form. If the reaction begins near or at the bottom it is called an UP reaction and conversely if the clearing reaction begins closer to the ball then that is a DO reaction. Time lapse images are taken every fifteen minute interval in the time lapse

camera until thirty hours and the CBR software upon request automatically determines the time lapse, predicts the population and gives the type of reaction. For convenience the data for the predicted bacterial populations is presented (Table 15.1) in predicted active cells per mL (pac/mL) and use billions (B), millions (M), thousands (T) while whole numbers are used where the population is less than one thousand.

For the ML WWTP the PI had an average of 22B pac/mL while the PI from the AL WWTP was 38.2B. The BOD5 for the rapid ML WWTP showed increases from an average of 194 to 842mg/L due to the greater amount of activity that occurred along with much higher amounts of E-tATP detected at those times. For AL WWTP the BOD5 declined from 229 to 31.4 (SE) and 9.9mg/L (FE) and for the ML WWTP the FE was 2.7mg/L. %CBR is given in Table 15.2 where the relationship between the BOD5 and %CBR.

Table 15.1 Statistical Comparison of the range in Bacteriological Populations using the CBR system for each of the stages in the WWTP that were subjected to simultaneous BOD5 Testing

		Bacteriological Population (pac/mL)					Count
	BOD5	max	av+	av	av-	min	
ML-FE	2.7	98.8M	35M	16.3M	-2.3M	685	102
AL-FE	9.9	467M	189M	73.5M	-41.6M	60.2T	84
AL-SE	31.4	2.45B	1.37B	807M	245M	89.1M	75
AL-PE	151	54.9B	39.1B	28.4B	17.6B	3.27B	75
ML-PI	194	70.7B	38.1B	22B	5.98B	4.41B	132
AL-PI	229	67.6B	49B	38.2B	27.3B	19.4B	132
ML-ML	842	70.7B	34.B	17.9B	916M	2.37B	69
						Total:	**669**

Note: ML, mixed liquor rapid treatment; AL, aerated lagoon treatment; FE, final effluent; SE, secondary effluent; PE, primary effluent; PI, primary influent; ML-ML, and mixed liquor phase of treatment; incubation was at 28±1^{o}C using the VBR II system

Table 15.2 Statistical Comparisons of the range in %CBR percentage reductions from PI in the Bacteriological Populations for the two (AL and ML) WWTP

		Percentage Confirmatory Bacteriological Reduction from PI					
	BOD5	**max**	**av+**	**av**	**av-**	**min**	**Count**
ML-PI*	194	0.00	0.00	0.00	0.00	0.00	132
ML-ML	842	78.1%	81.8%	-28.2%	-138%	-286%	23
ML-FE	2.7	100%	100%	99.86%	99.7%	99.2%	34
AL-PI*	229	0.00	0.00	0.00	0.00	0.00	84
AL-PE	151	76.7%	44.2%	25.0%	5.9%	-0.19	75
AL-SE	31	99.8%	99.2%	98.0%	96.9%	95.0%	75
AL-FE	9.9	100%	100.1%	99.8%	99.5%	98.8%	28

Total: 451

Note: BOD5 average for all readings in that row; PI data is taken as 0.00 since it has not been affected by the downstream treatment processes which could commonly cause a fractional decrease but for the mixed liquor (ML) from rapid WWTP there was there were occasions when the bacteriological populations increased and this is shown as a negative (-) in the data; incubation was at 28±1^{o}C using the VBR II system

From table 15.1 and 15.2 the average relationship could be seen between the BOD5, the bacterial populations and the %CBR. For the four stages of the AL WWTP (PI, PE, SE and FE) it was found that the average population dropped from 38.2B, to 28.4B (PE) and on down to 807M (SE) with the FE carrying 73.5Mpac/mL. The ML WWTP only had three principal stages (PI, ML, and FE) and the effects on the bacterial population was on average 22B (PI) dropping to 17.9M (ML) and then terminating in the FE at 16.3Mpac/mL. In this rapid treatment process the bacterial population did not significantly fall between PI and ML but the BOD5 increased on average from 194 to 842mg/L (a 330% increase due to the extreme treatment being imposed which reduced the treatment length significantly to two days. One clear event was the massive increase in activity (as represented by BOD5 and the E-tATP). Table 15.3 examines the relative occurrences of lower E-tATP values as this relates various treatment stages.

Table 15.3 Differentiation of the relationships between RBR (E-tATP, pg/mL) for the 842 samples used in the study from the various WWTP treatment stages.

	E-tATP >40,000pg/mL	**E-tATP 20,000 – 39,000**	**E-tATP 10,000 – 19,000**	**E-tATP <10,000**
AL PI (119)	112 (94%)	7 (6%)	0	0
AL PE (119)	115 (97%)	4 (3%)	0	0
AL SE (119)	106 (89%)	13 (11%)	0	0
AL FE (119)	6 (5%)	27 (23%)	45 (38%)	41 (34%)
ML PI (165)	145 (88%)	19 (16%	1 (1%)	0
ML ML (66)	66 (100%)	0	0	0
ML FE (135)	2 (1.5%)	3 (2%)	26 (19%)	104 (77%)

Note: Each box displays the number of sample fitting into that category and then the percentage (bracketed); sample source is shown in the left hand column together with the total number of samples tested in brackets; and a total 842 sample were included in this study; incubation was at $28\pm1^{o}C$ using the VBR II system

For the aerated lagoon WWTP the E-tATP was consistently above 20,000pg/mL in the PI, PE and SE with 94%, 97% and 89% above 40,000pg/mL. In the FE from the aerated lagoon WWTP 34% were below 10,000pg/mL and a further 38% were below 20,000 but above 10,000pg/mL. Thus there was a probability that 72% of the FE would be suitable for meeting BOD5 requirements as suitable for regulatory discharge using the threshold for compliance being at less than 20,000pg/mL. These remaining samples of AL FE rejected because of E-tATP results being greater than 20,000pg/mL would either be subjected to more rigorous testing, sent back for further treatment or held until the %CBR data has been generated (within 30hours). From table 15.2 the AL-FE using the %CBR was always better (with the 28 samples compared) than 98.8% with at least two thirds of the samples exceeding the requirement to have a reduced bacterial population from PI of 99.7% and an average BOD of 9.9mg/L. For the ML WWTP the average %CBR was 99.86% with the BOD5 being 2.7mg/L with the lowest %CBR being 99.2%. Thus the more effective ML WWTP at least 80% of the FE tested generated values that would allow unrestricted discharge meeting the BOD5 regulatory standards in thirty hours rather than the standard five days.

15.8 Summary of the proposed decision tree for %CBR validation of suitability for FE discharge with a low BOD5 potential.

Tentatively presented is the full decision tree covering the use of both the E-tATP and the %CBR measurements to determine equivalence is to BOD5 as being suitable for environmentally suitable discharge. It is recognized that the E-tATP test is completed within fifteen minutes of the sample being received and acclimatised to room temperature (22±3^{o}C). While the %CBR can be started at the same time data would not be generated completely until all of the testers had gone positive or 30 hours had elapsed at 28±1^{o}C. This decision tree is therefore set chronologically with the E-tATP data stream arriving before the %CBR.

1. From the E-tATP triplicated data for the sample under evaluation, was the value less than 10,000pg/mL; if YES got to 2 and if then NO go to 3.
2. Sample has a low potential BOD5 equivalence and can be accepted for discharge into the recognized suitable environment.
3. Is the E-tATP average of triplicated analysis >9,999 but <20,000pg/mL. If YES then go to 4; if NO go to 7.
4. Repeat E-tATP in triplicate and is the mean value was <10,000pg/mL. If YES then go to 2; if NO then go to 5.
5. Wait until the %CBR data is generated (no longer than 30 hours) and examine the data. Is the %CBR showing greater than 99.7% reduction from the original PI? If YES then go to 2; if NO then go to 6.
6. The data suggests that the treatment of the sanitary wastes is not complete and further treatment should be applied and then the whole testing protocol should be started, when completed go to 1.
7. Is the E-tATP greater then 20,000pg/mL but less than 40,000pg/mL. If YES then go to 8, if NO then go to 9.
8. Hold until the %CBR data is generated and is this greater than 99.7%? if YES then go to 2; if NO then go to 6.

15.9 Advantages in the %CBR testing protocols

This second generation testing employs the visual Bart reader system (VBR™) which has been designed to meet both goals. Precision is improved by the use of a time lapse camera that takes an image every fifteen minutes (recommended but adjustable) of eighteen lab testers set in two both bottom daylight LED-illuminated rows of nine. Using the VBR software using the folder of jpg images from a test run it is possible to save the reaction types observed and the predicted populations (as pac/mL). Where triplicate testing is performed then the precision of the predicted population is commonly within 5% of each other with a 6% possibility of an outlier sample that would give either an inordinately high or low value. For confirmation of any specific reaction observed then the VBR software can be moved into project mode by typing "droycon5851762" in the lower right box. This now allows the active screen to zoom in or out for the display of one or more to form a convenient selection of testers.

Labour costs are a serious concern when using the HAB- or BOD- Bart tester since these have to be read each day until the test goes positive (or remains negative at the end of the run). There are two time factors involved that do have a cost attached. These are: (1) time spent each working day to examine the testers individually; and (2) time spent at weekends and statutory holidays when an operator would have to come in to read the testers. Generally a Bart tester would take a maximum of two minutes to read and write any relative outcome. If the tester was to be read every day for seven days then that would directly and minimally involve ten minutes of work time and then four minutes of weekend time for a total of fourteen minutes. This would mean that each Bart tester would directly (minimally) involve a quarter of an hour plus additional time and costs at the weekend and statutory holidays.

The VBR system recognizes these concerns (precision and labour costs) by providing an automatic recording system (using a time lapse camera) that simplifies and improves precision when using Barts for testing. VBR II is a more advanced system (Plate 15.1) that employs an incubator to control the temperature. It is recommended that testing can be performed at temperatures of up to $62\pm1^{o}C$. Higher temperatures than $62^{o}C$ are not recommended for BART testing since higher temperatures can impact on the structural integrity of the tester. Plate 15.2 shows five VBR II units operating in a common setting.

Plate 15.1 VBR II, Advanced Temperature Controlled Unit

Height: 22”; 53cms / Width: 19”; 48cms / Depth: 33”; 84cms.
Minimal area: 6 square feet / Voltage: 110AC 0.7A Max / Lighting: LED 12V Max 4.86A, 58.5W
Incubator: Temperature adjustment from ambient to 62±1^{o}C, 110V 2.2A
Camera: Time lapse battery operated (4x AA) with at least 4GB memory card
Array of Lab BARTs: two rows of nine testers for a maximum of 18; note that field testers can also be monitored if the shelf is installed for the bottom row; top row will accommodate the testers as two rows of five (10 testers)
Software: VBR version 1301 (for most testers) and % CBR 13 (for the HAB/BOD testers only)
Camera time lapse: every fifteen minutes (recommended)
Photo-entrapment: both VBR1301 (all BART lab testers) and % CBR 13 (HAB- testers only) does allow taking .jpg images of the whole frame or selected parts when using software. CBR3 allow the detection of UP and DO reactions using the HAB- tester and will automatically calculate time lapse and population with options of turning data into Excel or word formats.
Time Lapse Camera Specifications is the TimelapseCam™ version 8 with a resolution of 8.0 Megapixels set with a photo resolution of 3,264 x 2,448 (MAX) with a 43^{o} lens (35mm equivalent of 59mm). Focus range from 8” to infinity. Camera is powered by four AA batteries. Picture count on a 4GB memory card would be 1,920 pictures and 16GB would allow a count of 7,800. The camera should be operated in AUTO mode which would allow timelapse operation. This camera is equipped with a daily wakeup and sleep function and it recommended that both should be set to the same time (e.g. MIDNIGHT) in which case that function is turned off. It is not recommended that rechargeable batteries be used in the camera and that the camera be not connected to a voltage converter for secured power unless an interrupted power supply is also used. Note never to run the camera with both the batteries in line as well as the external power supply.

Plate 15.2 VBR II, advanced temperature controlled units set up as five independent systems

Advantages

VBR systems offer the following advantages:

- Permanent record with stored images and interpretations available at any time and these can be sent over the internet (for example by e mail)
- Once set up the VBR is self-sustaining and does not need daily observations to obtain daily data since the time lapse camera automatically records (jpg) images every fifteen minutes (recommended).Additionally there is a time saving in that there is no need to have operators coming in on a daily basis to "read" the testers. This saves as much as ten minutes per tester or three hours for a fully charged VBR system operating for ten days. This does not include additional times associable with readings on weekends and statutory holidays.
- Interpretation of the VBR data can be undertaken at any time during the run and it is possible to screen the tester data (like a movie) and stop only at frames where a new reaction is first noticed. When this is done the reaction can be entered and this triggers the calculation of the time lapse and then (upon request) the population in pac/mL assuming that 15mL of sample has been used.
- Data can be downloaded to either a .txt file (for incorporation into word documents) or excel (.xls) files for storage and further comparative interpretations.
- Remaining battery life can be monitored after power up, set up and then when starting under auto at which time the percentage life remaining is displayed.

Limitation

VBR systems I and II are specifically designed to allow the simultaneous testing of up to eighteen lab testers at any time. Field testers can be used in either the VBR I or II systems to a maximum of ten (2 rows of 5) using the table supplied for the bottom row

Warranty

This is one year on the VBR unit itself but the camera (both VBR I and II) and incubator (VBR II only) are covered by the manufacturer's warranties for those components.

15.10 %CBR program management

%CBR program (percentage confirmatory bacterial reduction) function with both the VBR I and II systems to allow the interpretation of the jpg image saved in the time lapse sequence on the memory card in the camera during the data collection phase. Plate 15.3 (below) shows the display of (up to) eighteen testers racked within the incubator. To bring in the correct folder it is necessary to load the test image series (blue box). Clicking on this box opens up the computers directories to find the correct folder, open that folder and click on the first .jpg image in that folder. %CBR assumes that this .jpg image will be the first in the series that will then be displayed to the left showing the initial testers before any incubation (time zero). On, or close to, each tester are two vertical red circles. To allow calibration (red circles shown in each tester when requested for calibration and confirmation) it is necessary to confirm that the red circles are correctly positioned in the testers. If the red circles are not visible then click on the top left hand (shown as orange) square for the red circles to be displayed ready for calibration. When calibrated all of the red circles should be aligned in both rows to the centre of each tester. The upper red circle should be positioned just below the midpoint of the liquid column in the tester. Using the various buttons within the calibration (upper dark blue rectangle) the buttons can be used to attain the correct position using the up, down, zoom, left and right functions. When the circles are correctly aligned for all eighteen testers then clicking on save default (right hand orange square) will save the calibration code (shown lower part of the dark blue box) as the default. Once this has been set then that is the calibration for that that particular VBR system. Calibration can however be changed if required. To scroll through the images then use the curser (lower left) and move it (green vertical arrow) between the start to the left (broken green arrow

left) and right (broken green arrow right). Moving this curser creates a "movie" – like effect and as the .jpg images change so does the time lapse (now displayed in the on the upper left side of the dark blue square). It is now possible to determine the time at which each tester actually goes positive. In plate 15.3 the yellow dashed line passes laterally through the upper six testers to the right shows that these are all going positive with UP reactions. It is recommended for convenience that the primary influent (PI) be performed in triplicate in the upper three left hand slots and the final effluent (FE) triplicated testers are on the center three slots in the upper row. Analysis is done in clusters of three (triplicated). For example the lower mid red square shows the button (red arrow) that allows the analysis of that triplicate set of testers. To conduct the analysis click on the upper box and that will initiate a scan of all the testers until all three have been detected as positive. As the curser (green vertical arrow) moves across then positive UP and DO reactions are recognised and the predicted population is determined. When this has happened then that box will display a plus sign. If no reaction is detected then a negative reaction (-, not detected) is displayed. If the scan shows no tester to be in that slot then a diagonally crossed "0" is displayed. Once that scan is completed then the average population is projected (horizontal red arrow). When all of the testers have turned positive or at 30 hours the final data can be saved as word compatible text file (middle green broken arrow) or spreadsheet file in excel (right hand green broken arrow). An additional useful feature for the CBR software is the ability to save a .jpg image of any part of the testers' images clicking on the image and then moving the curser until it displays that image you would like to save. Clicking on the left hand curser (left hand green broken arrow) allows you to save the image as it is displayed in the left hand red square.

Data from the testing can be saved in three modes: (txt, xls, and jpg). All of the data is saved automatically when the program is closed (upper right white x on red). If there is any concern about a particular or groups of reactions then the relevant .txt, .xls or .jpg files can be sent via the internet for appraisal. %CBR (percentage confirmatory bacterial reduction) can also be calculated once the evaluation period is over (i.e. all testers are positive, or 30 hours has passed). Questions, concerns and independent assessments can be e mailed to sales@dbi.ca, by phone to 306 585 1762, or by fax to 306 585 3000 attention support at rapidbod.

Note that in 2013 Luminultra Technologies Ltd introduced some improvements to the ATP system making it more efficient with changes to both the process and the method of reading that data. The total ATP discussed in the book is the old methodology and the replacement is the

QuenchGone 21 Industrial (Q621I). Initial trials of the new luminometer (PhotonMaster™) have found that it has greater precision and convenience than the old reader system it replaced.

Plate 15.3 Operating screen for the CBR system showing controls (right and below) and the HAB- testers being observed (to the left)